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(54) **NOVEL POLYPEPTIDE**

(57) It is to provide agents for diagnosing, preventing and treating a disease related to JNK3 cascade.

The present invention provides a novel polypeptide JSAP which binds to JNK3, a production method of the polypeptide, DNA encoding the polypeptide, a recombinant DNA obtained by inserting the DNA into a vector,

a transformant comprising the recombinant vector, an antibody recognizing the polypeptide, a determination method and immunostaining method of the polypeptide of the present invention using the antibody, a screening method using the polypeptide, and agents for diagnosing, preventing and treating a disease related to JNK3 cascade using the polypeptide, the DNA or the antibody.

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Description**TECHNICAL FIELD**

5 **[0001]** The present invention relates to a novel polypeptide which binds to JNK3 which is an isozyme of c-Jun N-terminal kinase (JNK); a DNA encoding the polypeptide; a vector containing the DNA; a transformant transformed with the vector; and a method for producing the polypeptide.

10 **[0002]** Furthermore, the present invention relates to an antibody which recognizes the polypeptide; a microorganism, animal cell or animal which produces the antibody; a method of screening a compound having a JNK3 signal transduction inhibitory activity using the polypeptide, a part thereof or a microorganism, animal cell or the like expressing the polypeptide or a part thereof; and a method of screening a compound which regulates gene expression of the polypeptide using the cell.

BACKGROUND ART

15 **[0003]** The cascade by mitogen-activated protein kinase (MAPK) which has an important role as an intracellular signal transduction molecule is an intracellular signal transduction pathway universally existing in eucaryotic organisms ranging from yeast to human.

20 **[0004]** In vertebral animals, three kinds of MAPK: extracellular signal-regulated kinase (ERK), p38 and JNK/SAPK (c-Jun amino-terminal kinase/stress-activated protein kinase), and a large number of their activating factors have been identified, and the presence of various functionally different MAPK pathways has been revealed.

25 **[0005]** It is considered that JNK, among MAPK, is activated by various extracellular stress factors, such as tumor necrosis factor- α (TNF- α), interleukin-1 (IL-1), epidermal growth factor (EGF), endotoxin lipopolysaccharide (LPS), heat shock, ultraviolet light (UV), X-ray and the like, and apoptosis (cell death) is induced by its activation (*Science*, 270: 1326 (1995)).

30 **[0006]** That is, in cells exposed to stress, when the stress signal is transferred to small G-protein (such as Rho, Ras or the like), MEKK1 (MAPK kinase kinase 1) as a member of MAPK kinase kinase (MAPKKK) is activated, and the activated MEKK1 phosphorylates SEK1 (also called MKK4) as a member of MAPK kinase (MAPKK) to activate SEK1. The activated SEK1 phosphorylates JNK (MAPK), and the JNK is activated. It is believed that the activated JNK phosphorylates c-Jun which is one of the transcription factors in the cell nucleus, and the transcription activity of the phosphorylated c-Jun is accelerated, then apoptosis is induced in participation with AP-1, activating transcription factor 2 (ATF2) and the like. However, the process of this apoptosis in the nucleus is not well understood.

35 **[0007]** Apoptosis is induced when a neuronal cell, PC12, is cultured using a medium containing a nerve growth factor (NGF) as a proteinous nutrient factor of neuronal cells, and culturing is continued after removing NGF. It is known that increase in JNK activity is observed in apoptosis induction (*Science*, 270: 1326 (1995)).

[0008] It is known that, among three kinds of JNK (JNK1, JNK2, JNK3), JNK3 is expressed at particularly high level in the brain (*EMBO J.*, 15: 2760 (1996)).

40 **[0009]** Resistance to an attack caused by kainic acid (which is an excitatory amino acid receptor agonist) was confirmed in JNK3 knockout mice, and the apoptosis in the hippocampus CA3 region caused by kainic acid, which was found in wild type mice, was not found (*Nature*, 389: 885 (1997)). Based on this, it is considered that the neuronal protection function by the knockout of JNK3 molecule is caused by the disappearance of JNK3 pathway.

45 **[0010]** Neuronal cell death caused by apoptosis is reported in neurodegenerative diseases. That is, there are reports stating, for example, that neuronal cells undergoing apoptosis are frequently found in the hippocampus of Alzheimer's disease patients (*Experimental Neurology*, 133: 225 (1995)), that fragmentation of DNA and apoptosis-specific changes in nuclei are observed in the brain of Alzheimer's disease patients (*Neuroreport*, 5: 2529 (1994); *Neuroreport*, 6: 1053 (1995)), and that apoptosis of neurons in substantia nigra is observed in Parkinson's disease (*J. Neurol. Sci.*, 137: 120 (1996)).

50 **[0011]** While JNK3 shows high expression in the brain, JNK1 and JNK2 are expressed in almost all tissues. The c-Jun protein, ATF2 and Elk-1 (one of the transcription factors which control expression of the gene) are considered to be the phosphorylation substrate of JNK1 and JNK2 (*Nature*, 389: 865 (1997)).

[0012] Although JNK3 binds to the above phosphorylation substrates, since the binding affinity is weaker than that of JNK1 and JNK2 (*EMBO J.*, 15: 2760 (1996)), it is not clear whether they are true phosphorylation substrates of JNK3 in mammals.

55 **[0013]** Since the kinase activity of JNK3 can be measured using only the c-Jun protein as substrate and there are few reports in mammals about the binding protein which is considered to interact with JNK3, biochemical reactions using a protein which interacts with JNK3 and controls functions of JNK3 have been hardly studied.

[0014] As a polypeptide capable of binding to JNK3 other than the above phosphorylation substrates, JNK/SAPK-associated protein (JSAP1a) has recently been reported (1997 Annual Meeting of The Molecular Biology Society of

Japan (December)).

[0015] As described below, it is assumed that JSAP1a functions as a scaffold protein of the JNK3 pathway. As the scaffold protein, JIP-1 (JNK interacting protein-1) which binds to JNK1 and JNK2 is known (*Science*, 281: 1671 (1998)). Variant JIP-1b having a sequence in which 47 amino acids are inserted after the 557th amino acid residue of JIP-1 is also known. The cDNA sequences encoding JIB-1 and JIP-1b have been registered in GenBank data base (accession numbers: AF003115 and AF054611, respectively).

[0016] Furthermore, it is known that STE5 protein functions as a scaffold protein in the signal transduction pathway of a mating pheromone of yeast *Saccharomyces cerevisiae*.

[0017] That is, STES protein is a scaffold protein which binds to all of the kinases STE11 (MAPKKK), STE7 (MAPKK) and FUS/KSS1 (MAPK) that exist in a series of MAP-kinase pathway in which the pheromone transfers a signal after binding to its receptor, thus having a function of transferring the signal efficiently (*Genes Dev.*, 8: 313 (1994); *Cell*, 78: 499 (1994); *Proc. Natl. Acad. Sci., U.S.A.*, 91: 7762 (1994)).

DISCLOSURE OF THE INVENTION

[0018] An object of the present invention is to provide an agent for preventing or treating neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis and the like, amyotrophic diseases such as amyotrophic lateral sclerosis and the like, ischemic diseases, brain damage due to stroke, schizophrenia, depression, epilepsy, or various immunological and inflammatory diseases, using a novel polypeptide which binds to JNK3 in the JNK3 cascade activated in response to a signal which induces stress and apoptosis, a DNA encoding the polypeptide, an antibody which recognizes the polypeptide and the like.

[0019] Activation of the JNK cascade is considered to be one of the mechanisms which cause apoptosis of cells. In addition, in neurodegenerative disease such as Alzheimer's disease or Parkinson's disease, apoptosis can be exemplified as the mechanism of cell death.

[0020] Thus, intensive studies were conducted based on a belief that an agent for treating these neurodegenerative diseases and others could be obtained if the JNK3 pathway which is especially highly expressed in the brain could be inhibited and blocked, and that an agent capable of selectively inhibiting and blocking the JNK3 pathway would provide a therapeutic agent having fewer side effects. Accordingly, the present invention has been accomplished.

[0021] Specifically, the present invention relates to the following inventions of (1) to (39).

(1) A polypeptide comprising an amino acid sequence selected from the amino acid sequences represented by any one of SEQ ID NOS:10 to 16.

(2) A polypeptide comprising an amino acid sequence in which at least one amino acid has been deleted, substituted or added in an amino acid sequence selected from the amino acid sequences represented by any one of SEQ ID NOS:14 to 16 and is capable of binding to JNK3.

The number of amino acids which have been deleted, substituted or added is not particularly limited in the above; however, they are preferably from 1 to tens, particularly from 1 to several amino acids. Also, in order to bind the polypeptide comprising a deleted, substituted or added amino acid sequence to JNK3 in the same manner as the original polypeptide, it is preferred that it has a homology of at least 60% or more, generally 80% or more, particularly 95% or more, with the amino acid sequence of the original polypeptide. Hereinafter, the amino acid sequence in which at least one amino acid has been deleted, substituted or added means a polypeptide based on the definition described above.

The deletion, substitution or addition of an amino acid can be carried out by the well-known site-specific mutagenesis technique, and specifically, it can be carried out according to the methods described in *Molecular Cloning, A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press (1989) (hereinafter referred to as "*Molecular Cloning*"), *Current Protocols in Molecular Biology*, Supplement 1-38, John Wiley & Sons (1987-1997) (hereinafter referred to as "*Current Protocols in Molecular Biology*"), *Nucleic Acids Research*, 10: 6487 (1982); *Proc. Natl. Acad. Sci. USA*, 79: 6409 (1982); *Gene*, 34: 315 (1985); *Nucleic Acids Research*, 13: 4431 (1985); *Proc. Natl. Acad. Sci. USA*, 82: 488 (1985); *Proc. Natl. Acad. Sci. USA*, 81: 5662 (1984); *Science*, 224: 1431 (1984); PCT WO 85/00817 (1985); *Nature*, 316: 601 (1985); and the like.

(3) A DNA which encodes the polypeptide of (1) or (2).

(4) A DNA comprising a nucleotide sequence selected from the nucleotide sequences represented by any one of SEQ ID NOS:2 to 8.

(5) A DNA which hybridizes with a DNA comprising the nucleotide sequence represented by any one of SEQ ID NOS:6 to 8 under stringent conditions, and encodes a polypeptide capable of binding to JNK3.

The above "DNA which hybridizes with a DNA comprising the nucleotide sequence represented by any one of SEQ ID NOS:6 to 8 under stringent conditions, and encodes a polypeptide capable of binding to JNK3" means a DNA obtained by colony hybridization, plaque hybridization, Southern blot hybridization or the like using the DNA

described in (3) or (4) as a probe. Examples include DNA which can be identified by carrying out hybridization at 65°C in the presence of 0.7-1.0 M NaCl using a filter on which a DNA prepared from colonies or plaques is immobilized, and then washing the filter with 0.1x to 2x SSC (saline-sodium citrate) solution (the composition of 1x SSC comprises 150 mM sodium chloride and 15 mM sodium citrate) at 65°C.

The hybridization can be carried out according to methods described in experiment texts, such as *Molecular Cloning*, 2nd ed., *Current Protocols in Molecular Biology*, *DNA Cloning 1: Core Techniques, A Practical Approach*, Second Edition, Oxford University Press (1995) and the like.

Specific examples of DNA which can be hybridized include a DNA having a homology of at least 80% or more, preferably 95% or more, with a nucleotide sequence represented by SEQ ID NOS:1 to 8 wherein the homology is calculated using BLAST (*J. Mol. Biol.*, 215: 403 (1990)), FAST (*Methods in Enzymology*, 183: 63-69) or the like.

(6) A recombinant DNA obtained by inserting the DNA of any one of (3) to (5) into a vector.

(7) The recombinant DNA according to (6), which is a recombinant DNA selected from plasmid pcDNA3-S-JSAP1b, plasmid pcDNA3-S-JSAP1c, plasmid pcDNA3-S-JSAP4, plasmid pGAD10-JSAP5, and plasmid pcDNA3-His-S-JSAP5.

(8) A transformant comprising the recombinant DNA of (6) or (7).

(9) The transformant according to (8), which is a transformant selected from a microorganism, an animal cell, a plant cell, and an insect cell.

(10) The transformant according to (9), which is a microorganism belonging to the genus *Escherichia*.

(11) The transformant according to (10), wherein the microorganism belonging to the genus *Escherichia* is a microorganism selected from *Escherichia coli* JSAP1b/pcDNA3 (FERM BP-6567), *Escherichia coli* JSAP1c/pcDNA3 (FERM BP-6568), *Escherichia coli* JSAP4/pcDNA3 (FERM BP-6569), *Escherichia coli* JSAP5/pGAD10 (FERM BP-6570), and *Escherichia coli* JSAP5/pcDNA3 (FERM BP-6928).

(12) A method for producing the polypeptide of (1) or (2), comprising culturing the transformant of any one of (8) to (11) in a medium to produce and accumulate the polypeptide of (1) or (2) in the culture, and recovering the polypeptide from the culture.

(13) An oligonucleotide which is selected from an oligonucleotide comprising a sequence identical to continuous 5 to 60 bases in a nucleotide sequence in any one of the DNA's of (3) to (5) and the DNA comprising the nucleotide sequence represented by SEQ ID NO:5, an oligonucleotide comprising a sequence complementary to the oligonucleotide, and an oligonucleotide analogue of these oligonucleotides.

(14) The oligonucleotide according to (13), wherein the oligonucleotide analogue is selected from oligonucleotide analogues in which: a phosphodiester bond is converted into a phosphorothioate bond, a phosphodiester bond is converted into an N3'-P5' phosphoramidate bond, a ribose-phosphodiester bond is converted into a peptide-nucleic acid bond, uracil is substituted with C-5 propynyluracil, uracil is substituted with C-5 thiazoleuracil, cytosine is substituted with C-5 propynylcytosine, cytosine is substituted with phenoxazine-modified cytosine, ribose is substituted with 2'-O-propylribose, and ribose is substituted with 2'-methoxyethoxyribose.

(15) A method for detecting mRNA encoding the polypeptide of (1) or (2), comprising using the oligonucleotide of (13) or (14).

(16) A method for inhibiting expression of the polypeptide of (1) or (2), comprising using the oligonucleotide of (13) or (14).

(17) An antibody which recognizes the polypeptide of (1) or (2).

(18) A method for immunologically detecting the polypeptide of (1) or (2), comprising using the antibody of (17).

(19) A method for immunohistologically staining of the polypeptide of (1) or (2), comprising using the antibody of (17).

(20) An immunohistologically staining agent, comprising the antibody of (17).

(21) A method of screening a compound having an inhibitory activity on binding of a polypeptide to JNK3, comprising bringing the polypeptide into contact with JNK3 and a test sample, said polypeptide comprising an amino acid sequence selected from the amino acid sequences represented by any one of SEQ ID NOS:9 to 16 or a polypeptide comprising an amino acid sequence in which at least one amino acid has been deleted, substituted or added in an amino acid sequence selected from the amino acid sequences represented by any one of SEQ ID NOS:9 to 16 and being capable of binding to JNK3.

(22) A method of screening a compound having an inhibitory activity on phosphorylation of a polypeptide caused by activated JNK3, comprising bringing the polypeptide into contact with activated JNK3 and a test sample, said polypeptide comprising an amino acid sequence selected from the amino acid sequences represented by any one of SEQ ID NOS:9 to 16 or a polypeptide comprising an amino acid sequence in which at least one amino acid has been deleted, substituted or added in an amino acid sequence selected from the amino acid sequences represented by any one of SEQ ID NOS:9 to 16 and being capable of binding to JNK3.

(23) A compound obtained by the method of (21) or (22) or a pharmacologically acceptable salt thereof.

According to the description, the pharmacologically acceptable salt of a compound includes pharmacologically

acceptable acid addition salts, metal salts, ammonium salts, organic amine addition salts, amino acid addition salts and the like.

(24) A method of screening a compound capable of changing expression of a gene encoding a polypeptide, comprising bringing a cell which expresses the polypeptide into contact with a test sample, said polypeptide comprising an amino acid sequence selected from the amino acid sequences represented by any one of SEQ ID NOS:9 to 16 or a polypeptide comprising an amino acid sequence in which at least one amino acid has been deleted, substituted or added in an amino acid sequence selected from the amino acid sequences represented by SEQ ID NOS:9 to 16 and being capable of binding to JNK3.

(25) The method according to (24), wherein the expression of a gene is detected by the method of (15).

(26) The method according to (24), wherein the polypeptide is detected using the method of (18).

(27) A compound obtained by the method of any one of (24) to (26) or a pharmacologically acceptable salt thereof.

(28) An inhibitor of binding of a polypeptide and JNK3, wherein the polypeptide comprises an amino acid sequence selected from the amino acid sequences represented by any one of SEQ ID NOS:9 to 16 or a polypeptide comprising an amino acid sequence in which at least one amino acid has been deleted, substituted or added in an amino acid sequence selected from the amino acid sequences represented by any one of SEQ ID NOS:9 to 16 and being capable of binding to JNK3.

(29) An inhibitor of phosphorylation of a polypeptide by activated JNK3 a polypeptide, wherein the polypeptide comprises an amino acid sequence selected from the amino acid sequences represented by any one of SEQ ID NOS:9 to 16 or a polypeptide comprising an amino acid sequence in which at least one amino acid has been deleted, substituted or added in an amino acid sequence selected from the amino acid sequences represented by any one of SEQ ID NOS:9 to 16 and being capable of binding to JNK3.

(30) An agent for preventing neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis and the like, amyotrophic diseases, such as amyotrophic lateral sclerosis and the like, ischemic diseases, brain damage due to stroke, schizophrenia, depression, epilepsy, or various immunological and inflammatory diseases, comprising the polypeptide of (1) or (2).

(31) An agent for treating neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis and the like, amyotrophic diseases, such as amyotrophic lateral sclerosis and the like, ischemic diseases, brain damage due to stroke, schizophrenia, depression, epilepsy, or various immunological and inflammatory diseases, comprising the polypeptide of (1) or (2).

(32) An agent for preventing neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis and the like, amyotrophic diseases, such as amyotrophic lateral sclerosis and the like, ischemic diseases, brain damage due to stroke, schizophrenia, depression, epilepsy, or various immunological and inflammatory diseases, comprising the oligonucleotide of (13) or (14).

(33) An agent for treating neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis and the like, amyotrophic diseases, such as amyotrophic lateral sclerosis and the like, ischemic diseases, brain damage due to stroke, schizophrenia, depression, epilepsy, or various immunological and inflammatory diseases, comprising the oligonucleotide of (13) or (14).

(34) An agent for preventing neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis and the like, amyotrophic diseases, such as amyotrophic lateral sclerosis and the like, ischemic diseases, brain damage due to stroke, schizophrenia, depression, epilepsy, or various immunological and inflammatory diseases, comprising the antibody of (17).

(35) An agent for treating neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis and the like, amyotrophic diseases, such as amyotrophic lateral sclerosis and the like, ischemic diseases, brain damage due to stroke, schizophrenia, depression, epilepsy, or various immunological and inflammatory diseases, comprising the antibody of (17).

(36) A promoter DNA which controls transcription of a gene encoding the polypeptide of (1) or (2).

(37) A screening method of a compound capable of changing efficiency of transcription by the promoter DNA of (36), comprising bringing a test sample into contact with a transformant comprising a plasmid containing the promoter DNA and a reporter gene connected to the downstream of the promoter DNA; and measuring a translation product content of the reporter gene.

(38) The method according to (37), wherein the reporter gene is a gene selected from a chloramphenicol acetyltransferase gene, a β -galactosidase gene, a luciferase gene, and a green fluorescent protein gene.

(39) A compound obtained by the method of (37) or (38) or a pharmacologically acceptable salt thereof.

[0022] The present invention is explained in detail below.

<1> Acquisition of the DNA of the present invention and preparation of oligonucleotide

(1) Production of cDNA library

In order to produce a cDNA library, total RNA or mRNA is prepared from a suitable cell or tissue.

As the method for preparing total RNA, a guanidine thiocyanate-cesium trifluoroacetate method (*Methods in Enzymology*, 154: 3 (1987)), an acidic guanidine thiocyanate phenol chloroform (AGPC) method (*Analytical Biochemistry*, 162: 156 (1987); *Experimental Medicine*, 9: 1937 (1991)) and the like can be used.

As the method for preparing mRNA as poly(A)⁺ RNA from the total RNA, an oligo(dT)-immobilized cellulose column method (*Molecular Cloning*, 2nd ed.), an oligo-dT latex-aided method and the like can be used.

Also, mRNA can be prepared directly from a tissue or cell by using a kit, such as Fast Track mRNA Isolation Kit (manufactured by Invitrogen), Quick Prep mRNA Purification Kit (manufactured by Pharmacia) or the like.

Using the resulting total RNA or mRNA, a cDNA library is produced by a known method.

Examples of the cDNA library production method include methods described in *Molecular Cloning*, 2nd ed., *Current Protocols in Molecular Biology*, *DNA Cloning 1: Core Techniques, A Practical Approach*, 2nd ed., Oxford University Press (1995) and the like, methods using a commercially available kit, such as Superscript Plasmid System for cDNA Synthesis and Plasmid Cloning (manufactured by Gibco BRL) and ZAP-cDNA Synthesis Kit (manufactured by Stratagene) and the like.

Any of phage vectors, plasmid vectors and the like can be used as the cloning vector for producing cDNA library, so long as it can autonomously reproduce in *Escherichia coli* K12.

Specific examples include ZAP Express (manufactured by Stratagene, *Strategies*, 5: 58 (1992)), pBlue-script II SK(+) (*Nucleic Acids Research*, 17: 9494 (1989)), Lambda ZAP II (manufactured by Stratagene), λ gt10 and λ gt11 (*DNA Cloning, A Practical Approach*, 1: 49 (1985)), λ TriplEx (manufactured by Clontech), λ ExCell (manufactured by Pharmacia), pT7T318U (manufactured by Pharmacia), pcD2 (*Mol. Cell. Biol.*, 3: 260 (1983)), pUC18 (*Gene*, 33: 103 (1985)), pAMo (*J. Biol. Chem.*, 268: 22782-22787 (1993)), alias pAMoPRC3Sc (Japanese Published Unexamined Patent Application No. 336963/93), pGAD10 (manufactured by Clontech) and the like.

Any microorganism can be used as the host microorganism, so long as it belongs to *Escherichia coli*. Specific examples include *Escherichia coli* XL1-Blue MRF' (manufactured by Stratagene, *Strategies*, 5: 81 (1992)), *Escherichia coli* C600 (*Genetics*, 39: 440 (1954)), *Escherichia coli* Y1088 (*Science*, 222: 778 (1983)), *Escherichia coli* Y1090 (*Science*, 222: 778 (1983)), *Escherichia coli* NM522 (*J. Mol. Biol.*, 166: 1 (1983)), *Escherichia coli* K802 (*J. Mol. Biol.*, 16: 118 (1966)), *Escherichia coli* JM105 (*Gene*, 38: 275 (1985)), *Escherichia coli* SOLRTM Strain (manufactured by Stratagene), *Escherichia coli* LE392 (*Molecular Cloning*, 2nd ed.) and the like.

In addition to the cDNA library produced by the above method, a commercially available cDNA library can also be used.

Representative cDNA libraries include those derived from organs of human, cattle, mouse, rat, rabbit and the like which are available from Clontech, Life Tech Oriental, Health Science Research Resources Bank, Japan and the like.

(2) Acquisition of the DNA of the present invention cDNA clone having the DNA of the present invention can be obtained from the cDNA library produced in the above (1) by the following two-hybrid system using yeast.

A full length cDNA encoding JNK3, such as mouse JNK3 (*Nature Medicine*, 3: 89 (1997)), is inserted into a cloning vector containing a sequence encoding the GAL4 DNA binding domain, such as the sequence of pAS2-1 (manufactured by Clontech), and then introduced into yeast CG-1945 (manufactured by Clontech).

By the method of the above (1), a brain-derived cDNA is inserted into a cloning vector containing a sequence encoding the GAL4 transcription activation domain, such as the sequence of pGAD10 (manufactured by Clontech), to produce a cDNA library.

The cDNA library is introduced into the above JNK3-containing CG-1945 to obtain a transformant.

The CG-1945 is a reporter yeast strain having *HIS3* and *lacZ* genes which are under control of the GAL4 response sequence, and the *HIS3* and *lacZ* genes are expressed only when two proteins expressed from hybrid constructs of JNK3 and brain cDNA are bound. Thus, cDNA clone having the DNA of the present invention encoding a polypeptide capable of binding to JNK3 (JSAP: c-Jun N-terminal kinase/stress-activated protein kinase-associated protein) can be selected by selecting a strain from which histidine requirement has been removed (e.g., which grows on a histidine-free medium) and which has a β -galactosidase activity.

The transformant from which histidine requirement has been removed and which has β -galactosidase activity can be obtained in one step, but the transformant of interest can also be obtained by first selecting a transformant from which histidine requirement has been removed or a transformant which has β -galactosidase activity (primary positive clone), and then selecting a transformant from which histidine requirement has been removed and which has β -galactosidase activity (secondary positive clone).

The brain-derived cDNA fragment of interest is obtained by recovering the pGAD-derived plasmid by a known method from the resulting transformant of interest.

Using the resulting cDNA fragment as the probe and labeling the probe, e.g., with an isotope or fluorescence, the full length cDNA of interest can be obtained by colony hybridization, plaque hybridization (*Molecular Cloning*, 2nd ed.) or the like.

A nucleotide sequence of the DNA obtained by the above method can be determined by inserting the DNA fragment, directly or after digestion with a suitable restriction enzyme or the like, into a vector and analyzing it by a generally used nucleotide sequence analyzing method such as the dideoxy method of Sanger *et al.* (*Proc. Natl. Acad. Sci. USA*, 74: 5463 (1997)) or using nucleotide sequence analyzing apparatus manufactured by Perkin Elmer (373A DNA sequencer), LI-COR, Pharmacia or the like.

The DNA obtained by the above method includes DNA comprising the nucleotide sequence represented by any one of SEQ ID NOS:1 to 8.

Escherichia coli JSAP1b/pcDNA3 comprising plasmid pcDNA3-S-JSAP1b containing DNA of the nucleotide sequence represented by SEQ ID NO:2, *Escherichia coli* JSAP1c/pcDNA3 comprising plasmid pcDNA3-S-JSAP1c containing DNA of the nucleotide sequence represented by SEQ ID NO:3, *Escherichia coli* JSAP4/pcDNA3 comprising plasmid pcDNA3-S-JSAP4 containing DNA of the nucleotide sequence represented by SEQ ID NO:6 and *Escherichia coli* JSAP5/pGAD10 comprising plasmid pGAD10-JSAP5 containing DNA of the nucleotide sequence represented by SEQ ID NO:7 have been deposited on November 6, 1998, and *Escherichia coli* JSAP5/pcDNA3 comprising plasmid pcDNA3-His-S-JSAP5 containing DNA of the nucleotide sequence represented by SEQ ID NO:8 on November 2, 1999, in National Institute of Bioscience and Human Technology, Agency of Industrial Science and Technology, Higashi 1-1-3, Tsukuba-shi, Ibaraki, Japan (postal code 305-8566), as FERM BP-6567, FERM BP-6568, FERM BP-6569, FERM BP-6570, and FERM BP-6928, respectively.

Also, a DNA of interest derived from other tissue or other animal, such as human, can be obtained by selecting a DNA which hybridizes under stringent conditions with the DNA obtained by the above method.

The DNA of interest can also be prepared by chemical synthesis using a DNA synthesizer based on the nucleotide sequence information obtained above. Examples of the DNA synthesizer include a DNA synthesizer manufactured by Shimadzu using the thiophosphite method, a DNA synthesizer model 392 manufactured by Perkin Elmer using the phosphoramidite method and the like.

With regard to the novelty of the resulting nucleotide sequence, such can be confirmed by searching a nucleotide sequence data base, such as GenBank, EMBL, DDBJ or the like, using a homology searching program, such as BLAST or the like.

With regard to the novel nucleotide sequences, known genes having the homology can be searched by first converting them into an amino acid sequence and then searching an amino acid sequence data base, such as GenPept, PIR, Swiss-Prot or the like, using a homology searching program, such as FASTA, Frame-Search or the like.

(3) Preparation of the oligonucleotide of the present invention

Oligonucleotides, such as antisense oligonucleotides, sense oligonucleotides and the like, having a partial sequence of the DNA of the present invention can be prepared by a known method or the above DNA synthesizer using the DNA of the present invention or a DNA fragment thereof obtained above.

Examples of the oligonucleotide include DNA comprising a sequence which is the same as continuous 5 to 60 nucleotides in a nucleotide sequence of the above DNA, and DNA comprising a sequence complementary to the DNA. Specific examples include DNA comprising a continuous 5 to 60 nucleotides in a nucleotide sequence represented by SEQ ID NOS:1 to 8, and DNA having a sequence complementary to such DNA.

When the oligonucleotide is used as the sense primer and antisense primer, the above oligonucleotide in which melting point (T_m) and the number of nucleotides are not significantly different from each other is preferred.

Furthermore, analogues of the oligonucleotide can also be used as the oligonucleotide of the present invention.

Examples of the oligonucleotide analogues include oligonucleotide analogues in which: a phosphodiester bond is converted to a phosphorothioate bond, a phosphodiester bond is converted to an N3'-P5' phosphoramidate bond, ribose and a phosphodiester bond is converted to a peptide nucleic acid bond, uracil is substituted with C-propynyluracil, uracil is substituted with C-5 thiazoluracil, cytosine is substituted with C-5 propynylcytosine, cytosine is substituted with phenoxazin-modified cytosine, ribose is substituted with 2'-O-propylribose, ribose is substituted with 2'-methoxyethoxyribose and the like (*Cell Engineering*, 16: 1463 (1997)).

<2> Preparation of the polypeptide of the present invention

(1) Production of transformant

Methods described in *Molecular Cloning*, 2nd ed., *Current Protocols in Molecular Biology* and the like can be used to express the DNA of the present invention encoding JSAP obtained by the method described in <1> above and produce the polypeptide of the present invention.

Specifically, a recombinant vector to which the DNA of the present invention has been inserted into downstream of the promoter of a suitable expression vector is constructed, a transformant expressing the polypeptide of the present invention is obtained by introducing the vector into a host cell, and the transformant is cultured to produce the polypeptide of the present invention.

Any of bacteria, yeast, animal cells, insect cells, plant cells and the like can be used as the host cell, so long as it can express the gene of interest.

Examples of the expression vector include those which can autonomously replicate in the above host cell or which can be integrated into a chromosome and have a promoter at an operative position such that the DNA of the present invention can be transcribed.

When prokaryote, such as a bacterium or the like, is used as the host cell, it is preferred that the expression vector of the polypeptide gene of the present invention can autonomously replicate in the prokaryote and is a recombinant vector constructed with a promoter, a ribosome binding sequence, the DNA of the present invention and a transcription termination sequence. A promoter-controlling gene can also be utilized.

Examples of the expression vector include pBTrp2, pBTac1 and pBTac2 (all available from Boehringer Mannheim), pKK233-2 (manufactured by Pharmacia), pSE280 (manufactured by Invitrogen), pGEMEX-1 (manufactured by Promega), pQE-8 (manufactured by QIAGEN), pKYP10 (Japanese Published Unexamined Patent Application No. 110600/83), pKYP200 (*Agric. Biol. Chem.*, 48: 669 (1984)), pLSA1 (*Agric. Biol. Chem.*, 53: 277 (1989)), pGEL1 (*Proc. Natl. Acad. Sci. USA*, 82: 4306 (1985)), pBluescript II SK(-) (manufactured by Stratagene), pTrs32 (FERM BP-5408), pGHA2 (FERM B-400), pGKA2 (FERM BP-6798), pTerm2 (Japanese Published Unexamined Patent Application No. 22979/91, US 4686191, US 4939094, US 5160735), pGEX (manufactured by Pharmacia), pET (manufactured by Novagen), pSupex, pUB110, pTP5, pC194, pTrxFus (manufactured by Invitrogen), pMAL-c2 (manufactured by New England Biolabs) and the like.

With regard to the promoter, any promoter can be used, so long as it can express in a host cell, such as *Escherichia coli*, *Bacillus subtilis* and the like. Examples include promoters derived from *Escherichia coli*, phage and the like, such as *trp* promoter (*P_{trp}*), *lac* promoter (*P_{lac}*), *P_L* promoter, *P_R* promoter, T7 promoter and the like; SPO1 promoter; SPO2 promoter; *penP* promoter; and the like. Also, artificially designed and modified promoters, such as a promoter in which two *P_{trp}* are linked in series (*P_{trp}x2*), *tac* promoter, *lacT7* promoter, *letI* promoter or the like, can be used.

As the ribosome binding sequence, it is preferred to use a plasmid in which the space between Shine-Dalgarno sequence and the initiation codon is adjusted to a suitable distance (for example, 6 to 18 nucleotides).

The transcription termination sequence is not required for the expression of the DNA of the present invention. However, the transcription terminating sequence is preferably arranged at just downstream of the structural gene.

Examples of the host cell include microorganisms belonging to the genus *Escherichia*, the genus *Serratia*, the genus *Bacillus*, the genus *Brevibacterium*, the genus *Corynebacterium*, the genus *Microbacterium*, the genus *Pseudomonas* and the like. Specific examples include *Escherichia coli* XL1-Blue, *Escherichia coli* XL2-Blue, *Escherichia coli* DH1, *Escherichia coli* MC1000, *Escherichia coli* KY3276, *Escherichia coli* W1485, *Escherichia coli* JM109, *Escherichia coli* HB101, *Escherichia coli* No.49, *Serratia ficaria*, *Serratia fonticola*, *Serratia liquefaciens*, *Serratia marcescens*, *Bacillus subtilis*, *Bacillus amyloliquefaciens*, *Brevibacterium ammoniagenes*, *Brevibacterium immariophilum* ATCC 14068, *Brevibacterium saccharolyticum* ATCC 14066, *Corynebacterium glutamicum* ATCC 13032, *Corynebacterium glutamicum* ATCC 14067, *Corynebacterium glutamicum* ATCC 13869, *Corynebacterium acetoacidophilum* ATCC 13870, *Microbacterium ammoniaphilum* ATCC 15354, *Pseudomonas* sp. D-0110 and the like. Any method can be used in the method for introducing the recombinant vector, so long as it is a method for introducing DNA into the above host cell. Examples include a method using a calcium ion (*Proc. Natl. Acad. Sci. USA*, 69: 2110 (1972)), a protoplast method (Japanese Published Unexamined Patent Application No. 248394/88), an electroporation method (*Gene*, 17: 107 (1982); and *Molecular & General Genetics*, 168: 111 (1979)) and the like.

When yeast is used as the host cell, examples of expression vector include YEp13 (ATCC 37115), YEp24 (ATCC 37051), YCp50 (ATCC 37419), pHS19, pHS15 and the like.

Any promoter can be used so long as it can express in yeast. Examples include PHO5 promoter, PGK promoter, GAP promoter, ADH promoter, *gal 1* promoter, *gal 10* promoter, a heat shock polypeptide promoter, MF α 1 promoter, CUP 1 promoter and the like.

Examples of the host cell include yeast strains belonging to the genus *Saccharomyces*, the genus *Schizosaccharomyces*, the genus *Kluyveromyces*, the genus *Trichosporon*, the genus *Schwanniomyces* and

the like. Specific examples include *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Kluyveromyces fragilis*, *Trichosporon pullulans*, *Schwanniomyces alluvius*, *Pichia pastoris* and the like.

Any method can be used as the method for introducing the recombinant vector, so long as it is a method for introducing DNA into yeast. Examples include an electroporation method (*Methods in Enzymology*, 194: 182 (1990)), a spheroplast method (*Proc. Natl. Acad. Sci. USA*, 81: 4889 (1978)), a lithium acetate method (*Journal of Bacteriology*, 153: 163 (1983)) and the like.

When an animal cell is used as the host, examples of expression vector include pcDNA1/Amp (manufactured by Invitrogen), pcDNA1 and pcDM8 (*Nature*, 329: 840 (1987)), pAGE107 (Japanese Published Unexamined Patent Application No. 22979/91; *Cytotechnology*, 3: 133 (1990)), pREP4 (manufactured by Invitrogen), pAGE103 (*Journal of Biochemistry*, 101: 1307 (1987)), pAMo, pAMoA, pAS3-3 (Japanese Published Unexamined Patent Application No. 227075/90) and the like.

Any promoter can be used as the method for introducing the recombinant vector, so long as it can express in the animal cell. Examples include a promoter of IE (immediate early) gene of cytomegalovirus (CMV), an early promoter of SV40, a metallothionein promoter, a promoter of retrovirus, a heat shock promoter, SRY promoter and the like. Also, the enhancer of the IE gene of human CMV can be used together with the promoter.

Examples of the animal cell include mouse myeloma cell, rat myeloma cell, mouse hybridoma cell, human Namalwa cell, human Namalwa KJM-1 cell, human fetal kidney cell, human leukemic cell, African green monkey kidney cell, Chinese hamster CHO cell, HBT5637 (Japanese Published Unexamined Patent Application No. 299/88) and the like.

Examples of the mouse myeloma cell include SP2/0, NS0 and the like. Examples of the rat myeloma cell include YB2/0 and the like. Examples of the human fetal kidney cell include BALL-1 and the like. Examples of African green monkey kidney cell include COS-1, COS-7 and the like.

Any method can be used as the method for introducing the recombinant vector into an animal cell, so long as it is a method for introducing DNA into an animal cell. Examples include an electroporation method (*Cytotechnology*, 3: 133 (1990)), a calcium phosphate method (Japanese Published Unexamined Patent Application No. 227075/90), a lipofection method (*Proc. Natl. Acad. Sci. USA*, 84: 7413 (1987)), the method described in *Virology*, 52: 456 (1973), and the like.

When an insect cell is used as the host, the polypeptide can be expressed by a known method described in, for example, *Baculovirus Expression Vectors, A Laboratory Manual*, W.H. Freeman and Company, New York (1992), *Molecular Biology, A Laboratory Manual, Current Protocols in Molecular Biology, BioTechnology*, 6: 47 (1988) or the like.

Specifically, a recombinant gene transfer vector and baculovirus are co-transfected into an insect cell to obtain a recombinant virus in an insect cell culture supernatant, and then the insect cell is infected with the resulting recombinant virus to express of the polypeptide.

Examples of the gene transfer vector used in the method include pVL1392, pVL1393, pBlueBac111 (all manufactured by Invitrogen) and the like.

Examples of the baculovirus include *Autographa californica* nuclear polyhedrosis virus which infects insects of the family *Barathra* and the like.

Examples of the insect cell include *Spodoptera frugiperda* ovary cell, *Trichoplusia ni* ovary cell, *Bombyx mori* ovary-derived culturing cell and the like.

Examples of *Spodoptera frugiperda* ovary cell include Sf9 and Sf21 (*Baculovirus Expression Vectors, A Laboratory Manual*) and the like. Examples of *Trichoplusia ni* ovary cell include High 5 and BTI-TN-5B1-4 (manufactured by Invitrogen) and the like. Examples of the *Bombyx mori* ovary-derived culturing cell include *Bombyx mori* N4 and the like.

The method for co-transfecting the above recombinant gene transfer vector and the above baculovirus for the preparation of the recombinant virus include a calcium phosphate method (Japanese Published Unexamined Patent Application No. 227075/90), a lipofection method (*Proc. Natl. Acad. Sci. USA*, 84: 7413 (1987)) and the like.

As the gene expression method, a secreted protein production, a fusion protein expression and the like can be effected according to the method described in *Molecular Cloning*, 2nd ed., in addition to direct expression.

When the expression is carried out in yeast, an animal cell or an insect cell, a glycosylated polypeptide can be obtained.

The resulting transformant is cultured in a medium, the polypeptide of the present invention is formed and accumulated in the medium, and the resulting polypeptide is recovered to produce the polypeptide of the present invention.

Furthermore, a suitable expression vector for expressing the polypeptide of the present invention can be introduced into a cell obtained from the body of a patient, and then the cell is returned into the patient to

express the polypeptide of the present invention in the patient's living body.

(2) Culturing of the transformant

Culturing the transformant of the present invention in a medium is carried out according to a known method used in culturing of the host.

As a medium for culturing the transformant obtained by using, as the host, prokaryote (such as *Escherichia coli* or the like) or eukaryote (such as yeast or the like), the medium may be either a natural medium or a synthetic medium, so long as it contains a carbon source, a nitrogen source, an inorganic salt and the like which can be assimilated by the organism and the transformant can be cultured efficiently.

Any source can be used as the carbon source, so long as it can be assimilated by the organism. Examples include carbohydrates, such as glucose, fructose, sucrose, molasses containing them, starch, starch hydrolysate and the like; organic acids, such as acetic acid, propionic acid and the like; alcohols, such as ethanol, propanol and the like; and so on.

Examples of the nitrogen source include ammonia; ammonium salts of inorganic acids or organic acids, such as ammonium chloride, ammonium sulfate, ammonium acetate, ammonium phosphate and the like, other nitrogen-containing compounds, peptone, meat extract, yeast extract, corn steep liquor, casein hydrolysate, soybean meal and soybean meal hydrolysate, various fermented cells and hydrolysates thereof and the like.

Examples of the inorganic salt include potassium dihydrogen phosphate, dipotassium hydrogen phosphate, magnesium phosphate, magnesium sulfate, sodium chloride, ferrous sulfate, manganese sulfate, copper sulfate, calcium carbonate and the like.

Culturing is generally carried out under aerobic conditions by shaking culture, aeration stirring culture or the like. The culturing temperature is preferably from 15 to 40°C, and the culturing time is generally from 16 to 96 hours. The pH is maintained at 3.0 to 9.0 during the culturing. The pH is adjusted using inorganic or organic acid, an alkali solution, urea, calcium carbonate, ammonia or the like.

Also, antibiotics, such as ampicillin, tetracycline and the like, can be added to the medium during the culturing, if desired.

When a microorganism transformed with an expression vector using an inducible promoter as the promoter is cultured, an inducer can be added to the medium, if desired. For example, when a microorganism transformed with an expression vector using *lac* promoter is cultured, isopropyl-β-D-thiogalactopyranoside or the like can be added to the medium, and when a microorganism transformed with an expression vector using *trp* promoter is cultured, indoleacrylic acid or the like can be added to the medium.

Examples of the medium for culturing a transformant obtained using an animal cell as the host include generally used RPMI 1640 medium (*The Journal of the American Medical Association*, 199: 519 (1967)), Eagle's MEM (*Science*, 122: 501 (1952)), DMEM (*Virology*, 8: 396 (1959)), and 199 Medium (*Proceeding of the Society for the Biological Medicine*, 73: 1 (1950)), media to which fetal calf serum or the like has been added to these media and the like.

Culturing is generally carried out under conditions at pH of 6 to 8 and at 30 to 40°C for 1 to 7 days in the presence of 5% CO₂ or the like.

Furthermore, if desired, antibiotics, such as kanamycin, penicillin, streptomycin and the like, can be added to the medium during the culturing.

Examples of the medium for culturing a transformant obtained using an insect cell as the host include generally used TNM-FH medium (manufactured by Pharmingen), Sf-900 II SFM (manufactured by Life Technologies), ExCell 400 and ExCell 405 (both manufactured by JRH Biosciences), Grace's Insect Medium (*Nature*, 195: 788 (1962)) and the like.

Culturing is generally carried out under conditions at pH of 6 to 7 and at 25 to 30°C for 1 to 5 days or the like.

Furthermore, if desired, antibiotics, such as gentamicin and the like, can be added to the medium during the culturing.

(3) Isolation and purification of the expressed polypeptide

When the polypeptide of the present invention expressed by the above method is isolated and purified from the above culture medium of the transformant, usual methods for isolating and purifying enzymes can be used.

For example, when the polypeptide of the present invention is expressed in a dissolved state inside the cells, the cells after completion of the culturing are recovered by centrifugation, suspended in an aqueous buffer and then disrupted using an ultrasonic oscillator, a French press, a Manton Gaulin homogenizer, a dynamill or the like to obtain a cell-free extract.

A purified product can be obtained from a supernatant prepared by centrifuging the cell-free extract with a technique, such as solvent extraction; salting out with ammonium sulfate or the like; desalting; precipitation

with an organic solvent or the like; anion exchange chromatography using a resin, such as diethylaminoethyl (DEAE)-Sephacrose, DIAION HPA-75 (manufactured by Mitsubishi Chemical), etc.; cation exchange chromatography using a resin, such as S-Sepharose FF (manufactured by Pharmacia), etc.; hydrophobic chromatography using a resin, such as butyl-Sepharose, phenyl-Sepharose, etc.; gel filtration using a molecular sieve; affinity chromatography; chromatofocusing; electrophoresis, such as isoelectric focusing, etc.; or the like, alone or in combination.

Also, when the polypeptide is expressed inside the cells in the form of an inclusion body, the cells are recovered and then disrupted in the same manner, the polypeptide is recovered from the precipitate fraction obtained by centrifuging the cells, and then the inclusion body of the polypeptide is solubilized with a protein modifier.

The solubilized solution is diluted or dialyzed to a solution containing no protein denaturant or a solution containing such a low concentration of the polypeptide denaturant that the protein is not denatured, the polypeptide having a normal stereostructure is folded, and then a purified product is obtained by an isolation and purification method similar to the above.

When the polypeptide of the present invention or an analogue thereof, such as a glycosylated product or the like, is secreted extracellularly, the polypeptide of the present invention or the analogue thereof, such as the glycosylated product or the like, can be recovered from the culture supernatant.

That is, the soluble fraction is obtained by treating the culture by a technique, such as centrifugation or the like, in the same manner as the above, and a purified product can be obtained from the soluble fraction by an isolation and purification method similar to the above.

Furthermore, a fusion protein of the polypeptide of the present invention and other protein is produced, and it can be purified using affinity chromatography using a substance having affinity to the fusion protein. For example, a fusion protein of the present invention and protein A is produced according to the method of Lowe *et al.* (*Proc. Natl. Acad. Sci. USA*, 86: 8227 (1989); *Genes Develop.*, 4: 1288 (1990)), or the method described in Japanese Published Unexamined Patent Application No. 336963/93 or 823021/94, and it can be purified by affinity chromatography using immunoglobulin G.

Moreover, the polypeptide of the present invention is produced as a fusion protein with Flag peptide, and the fusion protein can be purified by affinity chromatography using an anti-Flag antibody. Further purification can be carried out by affinity chromatography using the antibody against the polypeptide *per se*.

Furthermore, the polypeptide of the present invention can be produced by a chemical synthesis method, such as Fmoc method (fluorenylmethyloxycarbonyl method), tBoc method (t-butyloxycarbonyl method) or the like.

Also, the polypeptide can be chemically synthesized using a peptide synthesizer manufactured by Advanced ChemTech, Perkin-Elmer, Pharmacia, Protein Technology Instrument, Synthecell-Vega, Shimadzu Corporation or the like.

The structure of the purified polypeptide of the present invention is determined by any method generally used in the field of protein chemistry, for example, the method described in *Protein Structure Analysis for Gene Cloning* (Hisashi Hirano, Tokyo Kagaku Dojin, 1993).

<3> Preparation of antibody which recognizes the polypeptide of the present invention

(1) Preparation of polyclonal antibody

A purified product of a full length or a partial fragment of the polypeptide obtained by the method described in the above <2> is used as an antigen, and administered to an animal to produce a polyclonal antibody.

The peptide used as the antigen can be produced by a peptide synthesizer based on the amino acid sequence of the polypeptide of the present invention.

A purified product of a full length or a partial fragment of the polypeptide obtained by phosphorylating the polypeptide obtained in the method described in the above <2> using activated JNK3 described below is used as an antigen, and administered to an animal to produce a polyclonal antibody which specifically recognizes the phosphorylated polypeptide of the present invention.

Examples of the phosphorylated polypeptide of the present invention include the amino acid sequence represented by SEQ ID NO:9 in which at least one of 234th, 244th and 255th amino acids has been phosphorylated, the amino acid sequence represented by SEQ ID NO:10 in which at least one of 243rd, 253rd and 264th amino acids has been phosphorylated, the amino acid sequence represented by SEQ ID NO:11 in which at least one of 266th, 276th and 287th amino acids has been phosphorylated, and the amino acid sequence represented by SEQ ID NO:12 in which at least one of 265th, 275th and 286th amino acids has been phosphorylated.

Examples of the animal to be administered include rabbits, goats, 3- to 20-week old rats, mice, hamsters

and the like.

The dose of the antigen is preferably 50 to 100 µg per animal.

When the peptide is used, the peptide which covalently binds to a carrier protein, such as keyhole limpet haemocyanin, bovine thyroglobulin or the like, is preferably used as the antigen.

The administration of the antigen is carried out 3 to 10 times at the intervals of 1 or 2 weeks after the first administration. Three to seven days after each administration, blood is collected from the venous plexus of the eyeground, and it is confirmed that the serum reacts with the antigen by the enzyme immunoassay (*Enzyme Immunoassay (ELISA)*, Igaku Shoin (1988), *Antibodies - A Laboratory Manual*, Cold Spring Harbor Laboratory (1988)) or the like.

A serum is obtained from a non-human mammal having a sufficient antibody titer against the antigen used for the immunization, and the serum is isolated and purified to obtain a polyclonal antibody.

The methods for the isolation and purification include centrifugation, salting out by 40-50% ammonium sulfate, caprylic acid precipitation (*Antibodies, A Laboratory manual*, Cold Spring Harbor Laboratory (1988)), and chromatography using a DEAE-Sepharose column, an anion exchange column, a protein A or G-column, a gel filtration column and the like, which are used alone or in combination.

(2) Preparation of monoclonal antibody

(2-1) Preparation of antibody-producing cell

Three to seven days after the antigen substance is finally administered to a rat having a serum showing a sufficient antibody titer, the rat spleen is excised.

The spleen is cut to pieces in MEM (manufactured by Nissui Pharmaceutical), loosened using a pair of forceps, followed by centrifugation at 1,200 rpm for 5 minutes, and the resulting supernatant is discarded.

The spleen cells in the precipitated fraction is treated with a Tris-ammonium chloride buffer (pH 7.65) for 1 to 2 minutes to eliminate erythrocytes and washed three times with MEM, and the resulting spleen cells are used as antibody-producing cells.

(2-2) Preparation of myeloma cells

An established cell line derived from a mouse or a rat is used as myeloma cells. Examples include 8-azaguanine-resistant mouse (BALB/c) myeloma cell lines, such as P3-X63Ag8-U1 (P3-U1) (*Curr. Topics in Microbiol. Immunol.*, 81: 1 (1978), *Eur. J. Immunol.*, 6: 511 (1976)), SP2/O-Ag14 (SP-2) (*Nature*, 276: 269 (1978)), P3-X63-Ag8653 (653) (*J. Immunol.*, 123: 1548 (1979)), P3-X63-Ag8 (X63) (*Nature*, 256: 495 (1975)) and the like. These cell lines were subcultured in a 8-azaguanine medium (medium in which, to a medium obtained by adding glutamine (1.5 mM), 2-mercaptoethanol (5×10^{-5} M), gentamicin (10 µg/ml) and fetal calf serum (FCS) (manufactured by CSL, 10%) to RPMI-1640 medium (hereinafter referred to as the "normal medium"), 8-azaguanine (15 µg/ml) has been further added) and cultured in the normal medium 3 or 4 days before cell fusion, and 2×10^7 or more of the cells are used for the fusion.

(2-3) Production of hybridoma

The antibody-producing cells obtained in (2-1) and the myeloma cells obtained in (2-2) are washed with MEM or PBS (disodium hydrogen phosphate: 1.83 g, sodium dihydrogen phosphate: 0.21 g, sodium chloride: 7.65 g, distilled water: 1 liter, pH: 7.2) and mixed to give a ratio of antibody-producing cells : myeloma cells = 5 to 10 : 1, followed by centrifugation at 1,200 rpm for 5 minutes, and the supernatant is discarded.

The cells in the resulting precipitated fraction were thoroughly loosened, 0.2 to 1 ml of a mix solution of 2 g polyethylene glycol-1000 (PEG-1000), 2 ml MEM and 0.7 ml dimethylsulfoxide (DMSO) per 10^6 antibody-producing cells is added to the cells under stirring at 37°C, and then 1 to 2 ml of MEM is further added thereto several times at 1 to 2 minute intervals.

After the addition, MEM is added to prepare a total amount of 50 ml.

The resulting prepared solution is centrifuged at 900 rpm for 5 minutes, and then the supernatant is discarded.

The cells in the resulting precipitated fraction were gently loosened and then gently suspended in 100 ml of HAT medium (the normal medium to which hypoxanthine (10^{-4} M), thymidine (1.5×10^{-5} M) and aminopterin (4×10^{-7} M) have been added) by drawing up into and discharging from a measuring pipette.

The suspension is poured into a 96 well culture plate at 100 µl/well and cultured at 37°C for 7 to 14 days in a 5% CO₂ incubator.

After culturing, a part of the culture supernatant is recovered, and a hybridoma which specifically reacts with an antigen used for the immunization is selected to obtain the above antibody-producing cells according to the enzyme immunoassay described in *Antibodies, A Laboratory manual*, Cold Spring Harbor Laboratory

Press, Chapter 14 (1998) or the like.

A specific example of the enzyme immunoassay is described below.

A full length or a partial fragment of the polypeptide of the present invention used as the antigen in the immunization is coated on a suitable plate, the hybridoma-culturing supernatant or a purified antibody described in (2-4) below is allowed to react therewith as the first antibody, an anti-rat immunoglobulin antibody labeled with biotin, an enzyme, a chemiluminescent substance, a radioactive compound or the like is allowed to react therewith as the second antibody, and a hybridoma which specifically reacts with the polypeptide of the present invention is selected as a hybridoma capable of producing the monoclonal antibody against the polypeptide of the present invention.

Cloning is repeated using the hybridoma twice by limiting dilution analysis first in HT medium (a medium in which aminopterin has been removed from HAT medium) and then using normal medium, and a hybridoma which has a stable and sufficient antibody titer is selected as a hybridoma capable of producing the monoclonal antibody of the present invention.

(2-4) Preparation of monoclonal antibody.

The hybridoma cells capable of producing the polypeptide of the present invention obtained in (2-3) are injected intraperitoneally into a 8- to 10-week-old mouse or a nude mouse intraperitoneally treated with 0.5 ml of 2,6,10,14-tetramethylpentadecane (pristane), followed by 2 weeks of feeding at 5×10^6 to 20×10^6 cells/animal. The hybridoma causes ascites tumor in 10 to 21 days.

The ascitic fluid is collected from the mouse or nude mouse, and centrifuged to remove solid contents.

A monoclonal antibody can be purified and isolated from the resulting supernatant according to the method similar to that used in the polyclonal antibody.

The subclass of the antibody can be determined using a mouse monoclonal antibody typing kit or a rat monoclonal antibody typing kit. The protein amount can be determined by the Lowry method or by calculation based on the absorbance at 280 nm.

<4> Screening method of useful medicament using novel polypeptide which can bind to JNK3

(1) Preparation of fusion polypeptide between tag polypeptide and the polypeptide of the present invention used for screening

1) Preparation of fusion polypeptide with thioredoxin-S-tag (hereinafter referred to as "Trx-S") peptide

A vector capable of expressing a Trx-S-JSAP fusion polypeptide can be prepared by inserting a full length or a partial length DNA of cDNA encoding the polypeptide capable of binding to JNK3 (JSAP) obtained in <1> above into the downstream of the Trx-S sequence of an expression vector containing the Trx-S sequence, such as pET32 (manufactured by Novagen). In the same manner, a vector capable of expressing a Trx-S-ATF2-JSAP fusion polypeptide can be prepared by inserting the same into the downstream of a Trx-S-ATF2 sequence of an expression vector containing the Trx-S-ATF2 sequence, such as pET32a (manufactured by Novagen).

The fusion polypeptide can be obtained using the resulting expression vector according to the method described in <2> above.

2) Preparation of fusion polypeptide with S-tag peptide

A vector capable of expressing an S-JSAP fusion polypeptide can be prepared by inserting a full length or a partial length DNA of cDNA encoding JSAP into the downstream of the S-tag sequence of an expression vector containing the S-tag sequence, such as an s-modified pcDNA3 in which the S-tag sequence has been inserted into pcDNA3 (manufactured by Invitrogen).

The fusion polypeptide can be obtained using the resulting expression vector according to the method described in <2> above.

3) Preparation of fusion polypeptide with GAL4AD peptide

A vector capable of expressing a GAL4AD-JSAP fusion polypeptide can be prepared by inserting a full length or a partial length DNA of cDNA encoding JSAP into the downstream of a GAL4AD sequence of an expression vector containing the GAL4AD sequence, such as pGAD10 (manufactured by Clontech).

The fusion polypeptide can be obtained using the resulting expression vector according to the method described in <2> above.

4) Preparation of fusion polypeptide with Flag peptide

A vector capable of expressing a Flag-JSAP fusion polypeptide can be prepared by inserting a full length or a partial length DNA of cDNA encoding JSAP into the downstream of a Flag sequence of an expression vector containing the Flag sequence, such as pFlag-CMV-2 (manufactured by Kodak) or Flag-modified pcDNA3 vector (manufactured by Invitrogen) in which a Flag tag sequence has been inserted into pcDNA3.

The fusion polypeptide can be obtained using the resulting expression vector according to the method described in <2> above.

5) Preparation of fusion polypeptide with glutathione S-transferase (hereinafter referred to as "GST")

A vector capable of expressing a GST-JSAP fusion polypeptide can be prepared by inserting a full length or a partial length DNA of cDNA encoding JSAP into the downstream of a GST sequence of an expression vector containing the GST sequence, such as pGEX or pGEX-3X (both manufactured by Pharmacia).

The fusion polypeptide can be obtained using the resulting expression vector according to the method described in <2> above. The fusion polypeptide can be purified using a Glutathione Sepharose 6B column (manufactured by Pharmacia).

6) Preparation of fusion polypeptide with Myc tag peptide

A vector capable of expressing a Myc-JSAP fusion polypeptide can be prepared by inserting a full length or a partial length DNA of cDNA encoding JSAP into the downstream of a Myc tag sequence of an expression vector containing the Myc tag sequence, such as a Myc-modified pcDNA3. Myc-modified pcDNA3 (manufactured by Invitrogen) is pcDNA3 modified by connecting a Myc tag code sequence to the upstream of the gene to express the protein to which the tag has been added.

The fusion polypeptide can be obtained using the resulting expression vector according to the method described in <2> above.

7) Preparation of fusion polypeptide with His-S-tag peptide

A vector capable of expressing a His-S-JSAP fusion polypeptide can be prepared by inserting a full length or a partial length DNA of cDNA encoding JSAP into the downstream of a His-S-tag sequence of an expression vector containing the His-S-tag sequence, such as a His-S-modified pcDNA3. His-S-modified pcDNA3 (manufactured by Invitrogen) is pcDNA3 modified by connecting a His-S tag code sequence to the upstream of the gene to express the protein to which the tag has been added.

The fusion polypeptide can be obtained using the resulting expression vector according to the method described in <2> above.

According to the methods of the above 1) to 7), fusion polypeptides with the polypeptides related to the MAP kinase cascade can be prepared in the same manner.

(2) Preparation of activated JNK3

A vector is prepared by inserting a DNA encoding the full length or a partial length of Flag-JNK3 prepared according to the method of <4>(1) above into pFlag-CMV-2.

Also, a DNA encoding a Δ MEKK1 (a peptide containing from the 1169th to 1488th amino acid residues of MEKK1 and constantly activated) is inserted into expression vector pEF-BOS.

COS-7 cells are transfected with both of the resulting expression vectors using TransIT-LT1 (manufactured by Mirus), and the resulting COS-7 cells are cultured according to a known method to temporally express the Flag-JNK3 fusion polypeptide and Δ MEKK1.

After culturing for 24 to 48 hours, the cells are dissolved in a buffer B (50 mM HEPES (pH 7.5), 150 mM NaCl, 1% NP-40, 10% glycerol, 2 mM $MgCl_2$, 1 mM EGTA, 20 mM β -glycerophosphate, 2 mM Na_3VO_4 , 1 mM PMSF, 0.2 mM DTT), and the activated Flag-JNK3 is purified using an anti-Flag antibody column (manufactured by Kodak).

The activated Flag-JNK3 can be stored in a buffer containing from 20 to 50% glycerin or a buffer containing no glycerin at -20°C to -80°C, and can be used by thawing prior to use.

(3) Screening system using JNK3 binding activity as marker

Since the JNK3 pathway can be inhibited by finding a compound which inhibits binding of JNK3 with each of JSAP obtained in <2> above, it is useful in preventing and treating a disease caused by the JNK3 pathway such as apoptosis or the like.

The method of screening a compound having an activity of inhibiting each of JSAP with JNK3 is explained

in detail below.

1) Screening system 1 (method using cultured cells)

Transfection of COS-7 cells with the S-JSAP expression vector obtained according to the method of <4>(1) above and the Flag-JNK3 expression vector is carried out using TransIT-LT1 (manufactured by Mirus), and the resulting COS-7 cells are cultured according to a known method in the presence or absence of a test compound to transiently express the S-JSAP and Flag-JNK3 fusion polypeptide.

After culturing for 24 to 48 hours, the cultured cells are dissolved in the buffer B, S-protein agarose is added thereto, and then S-JSAP and polypeptide which binds to S-JSAP are precipitated and recovered.

The recovered polypeptide fraction is separated by SDS-PAGE and transferred on membrane Immobilon-P (manufactured by Millipore).

Western blotting is carried out using the membrane and anti-Flag M5 monoclonal antibody (manufactured by Kodak) as the probe, and the polypeptide which binds to the antibody (Flag-JNK3) is visualized using an ECL detection system (manufactured by Amersham) to determine the amount of JNK3 bound to JSAP.

Accordingly, a compound which inhibits binding with JNK3 can be detected using as a control the case in which the test compound is not added.

Examples of the test sample include synthetic compounds, natural proteins, artificially synthesized proteins, peptides, saccharides, lipids and modified products and derivatives thereof, as well as urine, body fluids, tissue extracts, cell culture supernatants and cell extracts of mammals (e.g., mouse, rat, guinea pig, hamster, swine, sheep, bovine, horse, dog, cat, monkey, human and the like), and also non-peptide compounds, fermentation products, extracts of plants and other organisms and the like. In the screening method described below, the test sample similar to these can be used.

2) Screening system 2 (ELISA)

The GST-JSAP fusion polypeptide prepared according to the method in <4>(1) above as such, or a JSAP polypeptide fragment obtained by digesting it with a protease factor Xa (manufactured by Sigma), is used in the binding reaction. Hereinafter, these polypeptides are called JSAP-related polypeptides.

The polypeptides can be stored at -80°C to -20°C in a buffer containing from 20 to 50% of glycerin or a buffer containing no glycerin, which can be used by thawing prior to use.

Each of the above JSAP-related polypeptides is added to a 96 well plate.

The activated JNK3 prepared in <4>(2) is (or the activated JNK3 and a test compound are) added to a buffer such as a binding buffer (50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.5% NP-40), followed by stirring. The thus stirred solution is added to the above plate.

After the addition, the plate is incubated at 4°C for 1 to 2 hours.

After incubation, the plate is washed three times with the same buffer, and the remaining activated JNK3 is determined by ELISA.

For example, the amount of the remaining activated JNK3 can be determined by ELISA using Phospho-SAPK/JNK(Thr183/Tyr185) antibody (manufactured by New England Biolabs) capable of recognizing the activated JNK3 as the first antibody, and using an antibody capable of recognizing the above antibody as the second antibody.

By the method, a compound which inhibits binding with JNK3 can be detected using as a control the case in which the test compound is not added.

(4) Screening system using phosphorylation of JSAP1 by JNK3 as marker

Because of the information regarding a) to c) obtained by Examples described below, establishment of a method for screening a compound having an inhibitory activity on phosphorylation of JSAP1 is important in detecting a compound useful in preventing and treating a disease caused by the JNK3 pathway.

a) Inactivated JNK3 is bound to scaffold polypeptides JSAP1a, b, c and d which are selectively expressed in the brain.

b) When the JNK3 pathway is activated (MAPKKK → MAPKK → JNK3) by various extracellular stresses, such as THF-α, IL-1, EGF, LPS and the like, JNK3 per se is activated (phosphorylated), and the activated JNK3 phosphorylates JSAP1a, b, c and d.

c) After the phosphorylation of JSAP1, JNK3 is released from JSAP1 and transferred into the nucleus.

It is believed that the JNK3 transferred into the nucleus activates various transcription factors and leads the cell into apoptosis and the like. Thus, a compound having an inhibitory activity on phosphorylation of JSAP1

by this JNK3 can inhibit the JNK3 pathway and therefore is useful in preventing and treating a disease caused by the JNK3 pathway, such as apoptosis or the like.

A method for screening a compound having an inhibitory activity on phosphorylation of JSAP1 by JNK3 is described below in detail.

1) Screening system (cell-free system)

1)-1 Screening system 1 (method using radioisotope)

A screening test solution is prepared by adding (i) GST-JSAP1 prepared according to the method described in <4>(1) or JSAP1 and (ii) the activated JNK3 prepared according to the method described in <4>(2) or activated JNK3 and a test compound to a buffer, such as a buffer A (20 mM HEPES, 10 mM MgCl₂, 5 mM β-mercaptoethanol, 0.1 mg/ml BSA (pH 7.4)) and mixing them. The screening test solution is added to a 96 well plate.

After the addition, [γ -³²P]ATP or [γ -³³P]ATP is added to the plate. After the reaction for 5 to 30 minutes, 50 mM EDTA is added to stop the reaction.

After termination of the reaction, the polypeptide in the reaction solution is trapped by vacuum filtration on the membrane of each well of a 96 well nitrocellulose membrane plate, a 96 well phosphocellulose membrane plate or a 96 well PVDF membrane plate, and the membrane is washed by aspiration with the same buffer.

The membrane is put into a liquid scintillator MicroScint-20 (manufactured by Packard) and the ³²P or ³³P radioactivity on the membrane is counted using Top Count (manufactured by Packard).

By the method, a compound having an inhibitory activity on phosphorylation of JSAP1 by JNK3 can be detected using as a control the count of a case in which the test compound is not added.

1)-2 Screening system 2 (ELISA)

An antibody (first antibody) which recognizes JSAP1 obtained according to the method described in <3> is coated on a 96 well plate.

To a screening test solution prepared according to the method described in 1)-1, 50 μM of ATP is added.

After the addition, the reaction is carried out at room temperature for 5 to 60 minutes, and the reaction is terminated with 0.25 N HCl.

After the termination, the reaction solution is neutralized with a solution containing 0.25 N NaOH and 0.1 M Tris-HCl (pH 8.0).

An aliquot of the neutralized solution is added to the above plate coated with the first antibody and allowed to stand.

After the standing, the plate is washed with the same buffer, and then an antibody (second antibody) which recognizes the phosphorylated JSAP1 obtained according to the method described in <3> is added thereto.

By determining the amount of the second antibody by ELISA in a known method, the amount of the phosphorylated JSAP1 is determined.

By the method, a compound having an inhibitory activity on phosphorylation of JSAP1 by JNK3 can be detected using as a control the amount of phosphorylated JSAP1 in a case in which the test compound is not added.

Although the compound which inhibits binding of the polypeptide of the present invention with JNK3 or the compound having the inhibitory activity on phosphorylation of JSAP1, obtained by the methods in the above 1)-1 and 1)-2, can be used directly as a therapeutic agent, generally, it is preferred to use the compound as a pharmaceutical preparation produced by a well-known method in the technical field of pharmaceuticals by mixing it with at least one pharmaceutically acceptable carrier.

As the administration method of the therapeutic agent, it is preferred to use the most effective method in carrying out the treatment, and methods by oral administration or by parenteral administration, such as buccal, airway, rectal, subcutaneous, intramuscular, intravenous or the like, can be used.

Examples of dosage form of the therapeutic drug include ointments, sprays, capsules, tablets, granules, syrups, emulsions, suppositories, injections, tapes and the like.

Examples of preparations suitable for oral administration include emulsions, syrups, capsules, tablets, powders, granules and the like.

Liquid preparations such as emulsions and syrups can be produced using, as additives, water; saccharides, such as sucrose, sorbitol, fructose, etc.; glycols, such as polyethylene glycol, propylene glycol, etc.; oils, such as sesame oil, olive oil, soybean oil, etc.; antiseptics, such as p-hydroxybenzoates, etc.; flavors, such as strawberry flavor, peppermint, etc.; and the like.

Capsules, tablets, powders, granules and the like can be produced using, as additive, fillers, such as

lactose, glucose, sucrose, mannitol, etc.; disintegrants, such as starch, sodium alginate, etc.; lubricants, such as magnesium stearate, talc, etc.; binders, such as polyvinyl alcohol, hydroxypropylcellulose, gelatin, etc.; surfactants, such as fatty acid ester, etc.; plasticizers, such as glycerin, etc.; and the like.

Examples of the preparations suitable for parenteral administration include injections, suppositories, sprays and the like.

The injections can be prepared using, for example, a carrier comprising a salt solution, a glucose solution, or a mixture of both or the like.

The suppositories can be produced using, for example, a carrier, such as cacao butter, hydrogenated fat, carboxylic acid or the like.

As the sprays, the phosphorylation inhibitor or binding inhibitor obtained in the foregoing can be used directly as sprays, but sprays prepared using a carrier or the like which does not stimulate buccal and airway mucous membranes of the recipient and can facilitate absorption of the compound by dispersing it as fine particles are preferred.

Examples of the carrier include lactose, glycerol and the like.

Depending on the properties of the agonist or antagonist obtained in the foregoing and of the carrier, it is possible to prepare pharmaceutical preparations, such as aerosols, dry powders and the like.

The components exemplified as additive agents of oral preparations can also be added to these parenteral preparations.

The dose or frequency of administration varies depending on the intended therapeutic effect, administration method, treating period, age, body weight and the like, but is generally from 10 µg/kg to 8 mg/kg per day and per adult.

<5> Screening and identification of compound which controls expression of the polypeptide of the present invention (hereinafter referred to as "expression-controlling compound")

(1) Screening and identification of expression-controlling compound using the antibody of the present invention

After allowing a cell expressing the polypeptide of the present invention to contact with a test sample, an expression-controlling compound existing in the cell or a cell culture supernatant can be screened and identified using the antibody of the present invention.

As the cell, any one of cells, cell lines and tissues which express the polypeptide of the present invention can be used.

Also, a cell, a cell line or tissue in which expression of the polypeptide was observed using the method for the immunological detection with the antibody described in <3> can be used.

As a suitable cell line, a mouse-derived P19 cell (ATCC CRL-1825) which is differentiated to be a neuronal cell like with retinoic acid is exemplified.

As the test sample, those which were exemplified in <4> above can be used.

Cells expressing the polypeptide of the present invention are suspended in a medium in which the cells can grow, each test sample is added to the medium for contact with the cells, and then the amount of the polypeptide expressed in the cells is determined using the antibody of the present invention. As the determination method using the antibody, the immunocytochemistry described below is exemplified.

Cultured adherent cells are washed with PBS, 3 ml of PBS containing 0.05% of trypsin and 0.02% of ethylenediaminetetraacetic acid (EDTA) was added thereto, and then, after removing an extra solution, incubated at 37°C for 5 minutes to peel off the cells from the flask.

The suspension cells can be used as cultured cells, as they are.

Cells used in the immunocytochemistry are suspended in a buffer for immunocytochemistry (PBS containing 1% BSA, 0.02% EDTA and 0.05% sodium azide) or the like and dispensed into a round bottom 96-well plate at 1×10^5 to 20×10^5 cells per well.

The monoclonal antibody of the present invention is dispensed into the plate.

As the monoclonal antibody, culture supernatant of the hybridoma obtained in <3>(2-3) capable of producing the monoclonal antibody of the present invention and the purified monoclonal antibody obtained in <3>(2-4) can be exemplified. Additionally, an antibody prepared by labeling the monoclonal antibody can be used.

As the antibody prepared by labeling the monoclonal antibody, a biotin-labeled antibody can be exemplified.

The biotin-labeled antibody can be prepared by a known method (*Enzyme Antibody Method*, published by Gakusai Kikaku (1985)).

The above antibody is diluted to a concentration of 0.1 to 50 µg/ml using the buffer for immunocytochemistry or the buffer for immunocytochemistry containing 10% animal serum.

The diluted antibody is dispensed to give an amount of 20 to 500 µl/well and incubated on ice for 30

minutes.

When the unlabeled antibody is used, the cells are washed by adding the buffer for immunocytochemistry to the above plate, the buffer for immunocytochemistry containing about 0.1 to 50 µg/ml of an anti-mouse immunoglobulin antibody or anti-rat immunoglobulin antibody labeled with fluorescence dye, such as fluorescein isothiocyanate (FITC), phycoerythrin or the like, is dispensed at about 50 to 500 µl/well, and then the cells are incubated on ice in the dark for 30 minutes.

When the monoclonal antibody labeled with biotin is used, streptavidin labeled with a fluorescence dye, such as FITC, phycoerythrin or the like, is dispensed at about 50 to 500 µl/well into the above plate, and then incubated on ice in the dark for 30 minutes.

In both cases, after incubation, the cells are thoroughly washed by adding the buffer for immunocytochemistry to the plate, and then analyzed by a fluorescence microscopy, a cell sorter or the like.

The analyzed result obtained by carrying out the same procedure without adding test samples is compared with the analyzed result obtained by adding test samples, and a test sample capable of increasing or decreasing the amount of the polypeptide of the present invention is screened and thereby identified as an expression-controlling compound.

(2) Screening and identification using transcription product determination system of the gene of the polypeptide of the present invention

An expression-controlling compound can be screened and identified by allowing cells which express the polypeptide of the present invention or mRNA encoding the polypeptide to contact with each test sample, and then determining the amount of the mRNA.

Cells which express the polypeptide of the present invention or mRNA encoding the polypeptide are suspended in a medium in which the cells can grow, each test sample is added to the medium for contact with the cells, and then the amount of the mRNA expressed by the cells is determined using a known Northern hybridization, RNA dot blot hybridization, RT-PCR or the like.

As probes which can be used in the hybridization and the like and primers which can be used in the RT-PCR and the like, fragments of the gene which encode the polypeptide of the present invention can be exemplified.

Specifically, an oligonucleotide having a sequence identical to continuous 5 to 60 bases in the nucleotide sequence represented by SEQ ID NOS:1 to 8 or an oligonucleotide having a sequence complementary to the oligonucleotide can be preferably used.

The determination result obtained by carrying out the same procedure without adding test samples is compared with the determination result obtained by adding test samples, and a test sample capable of increasing or decreasing amount of mRNA encoding the polypeptide of the present invention is screened and thereby identified as an expression-controlling compound.

(3) Screening and identification using reporter gene

A cell transformed with a plasmid containing a DNA in which a reporter gene is connected to the downstream of a region which controls transcription of a gene which encodes the polypeptide of the present invention (hereinafter referred to as "transcription-controlling region") is allowed to contact with each test sample, and then an expression-controlling compound can be screened and identified by determining the amount of the expressed polypeptide encoded by the reporter gene.

In general, the transcription-controlling region is contained in the 5' upstream of genes in most cases. The 5' upstream region of the gene encoding the polypeptide of the present invention can be prepared by, for example, using Genome Walker kits (manufactured by Clontech) or the like. Also, a fragment obtained by digesting the region into a suitable length using suitable restriction enzymes can be used as the transcription-controlling region.

Any gene can be used as the reporter gene, so long as a translation product of the gene is stable inside the cells and the existing amount of the translation product can easily be determined. Examples include chloramphenicol acetyltransferase (CAT), β-galactosidase (β-gal), luciferase (luc), green fluorescent protein (GFP) and the like.

As the host cell into which a reporter plasmid containing the transcription-controlling region is introduced, any cell can be used; however, a cell line in which expression of the polypeptide of the present invention or mRNA encoding the polypeptide is confirmed as described in <5>(1) is preferably used.

As the test samples, those described in <4> above can be used.

The host cell is transformed by a known method using a plasmid prepared by connecting the reporter gene to the downstream of the transcription-controlling region by a known method.

Alternatively, a cell line in which a part of the gene encoding the polypeptide of the present invention is

substituted with a reporter gene can be obtained by preparing a gene targeting vector in which a positive selection marker (G418 resistance gene or the like) and a negative selection marker (thymidine kinase gene of herpes simplex virus, diphtheria toxin A fragment gene or the like) are connected (*Nature*, 336: 348 (1988); *Analytical Biochemistry*, 214: 77 (1993); *Gene Targeting, The Practical Approach Series*, IRL Press (1993)).

The transformant cells are suspended, for example, in a medium in which the cells can grow, each test sample is added to the medium for contact with the cells, and then the amount of the polypeptide encoded by the reporter gene expressed by the cells is detected and determined using a method suitable for the polypeptide.

Examples of the detection and determination method include the method described in, for example, *Molecular Cloning*, 2nd ed., Chapter 16, p. 60 in the case of CAT; the method described in, for example, *Molecular Cloning*, 2nd ed., Chapter 16, p. 66 in the case of β -gal; the method described in, for example, *Experimental Medicine, Supplement, Biomanual Series 4, Gene Introduction and Expression Analysis Method*, 81 (1994) in the case of luc; the method described in, for example, *Proc. Natl. Acad. Sci. USA*, 94: 4653 (1997) in the case of GFP; and the like.

The determination result obtained by carrying out the same procedure without adding test samples is compared with the determination result obtained by adding test samples, and a test sample capable of increasing or decreasing amount of the polypeptide encoded by the reporter gene is searched and thereby identified as an expression-controlling compound.

<6> Use of the DNA, polypeptide, antibody, binding inhibitor, phosphorylation inhibitor and expression-controlling compound of the present invention

(1) The DNA of the present invention can be used for detecting or determining mRNA of the gene of the polypeptide of the present invention in human tissues and human-derived cells by carrying out Northern hybridization of RNA extracted from the tissues and cells in the same manner as described in <1>(1) using the DNA as the probe. Expression distribution of the polypeptide of the present invention in tissues can be known by comparing the expression amounts of mRNA in various tissues.

(2) The oligonucleotide of the present invention can be used for detecting and determining mRNA encoding the polypeptide of the present invention by carrying out RT-PCR (reverse transcription PCR; *PCR Protocols* (1990)) of RNA extracted from human tissues and human-derived cells in the same manner as described in <1>(1) using the oligonucleotide of the present invention as a specific primer of the DNA of the present invention.

The method for determining mRNA can be used in the diagnosis of disease states related to the gene.

Importance of the gene product in disease states can be revealed by determining the mRNA in animal models of various disease states. Also, medicaments can be evaluated by comparing expression amounts of the mRNA in the presence or absence of each medicament.

(3) More definite expression distribution, such as specification of the expressing cells of the polypeptide of the present invention in tissues, can be known by carrying out in situ hybridization (*Methods in Enzymology*, 254: 419 (1995)) of tissue sections of human using the oligonucleotide of the present invention as the probe.

Information concerning which tissues and cells express the polypeptide of the present invention and information concerning what kind of stimulation changes the expression level in cells are useful in analyzing participation of the polypeptide of the present invention in physiological functions and disease states.

(4) Mutation of the gene encoding the polypeptide of the present invention can be detected by carrying out Southern hybridization (*Molecular Cloning*, 2nd ed.) on genomic DNA using the DNA of the present invention as the probe.

Neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis and the like, amyotrophic diseases, such as amyotrophic lateral sclerosis and the like, ischemic diseases, brain damage due to stroke, schizophrenia, depression, epilepsy, or various immunological and inflammatory diseases, which have a possibility that mutation of the gene is a cause thereof, can be diagnosed by detecting the mutation.

(5) Neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis and the like, amyotrophic diseases, such as amyotrophic lateral sclerosis and the like, ischemic diseases, brain damage due to stroke, schizophrenia, depression, epilepsy, or various immunological and inflammatory diseases, in which the gene encoding the polypeptide of the present invention is involved

In the onset of diseases, can be prevented or treated by inhibiting transcription of the gene encoding the polypeptide of the present invention or translation of the mRNA (*Chemistry*, 46: 681 (1991); *Bio/Technology*, 9: 358 (1992)) using the antisense oligonucleotide (RNA/DNA) of the present invention.

The above antisense oligonucleotide is administered to the living body by designing and preparing it based on an oligonucleotide having a sequence complementary to continuous 5 to 60 bases in the nucleotide sequence of the DNA of SEQ ID NOS:1 to 8 encoding the polypeptide of the present invention, preferably a nucleotide sequence complementary to 5 to 60 bases existing in the translation initiation region of the DNA encoding the polypeptide of the present invention.

The medicament containing the DNA of the present invention can be prepared using a method similar to the preparation method of pharmaceutical preparations of the phosphorylation inhibitor or binding inhibitor of the polypeptide of the present invention described in <4> above, and the thus prepared pharmaceutical preparations can be administered in the same manner as in <4> above.

(6) The polypeptide of the present invention can be obtained by the method described in <2> using the DNA of the present invention.

An agent for treating or preventing neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis and the like, amyotrophic diseases, such as amyotrophic lateral sclerosis and the like, ischemic diseases, brain damage due to stroke, schizophrenia, depression, epilepsy, or various immunological and inflammatory diseases is considered as the use of the polypeptide of the present invention.

The medicament containing the polypeptide of the present invention can be prepared using a method similar to the preparation method of pharmaceutical preparations of the phosphorylation inhibitor or binding inhibitor of the polypeptide of the present invention described in <4> above, and the thus prepared pharmaceutical preparations can be administered in the same manner as in <4> above.

(7) The oligonucleotide of the present invention can be used in the gene therapy as a vector for gene therapy by inserting it, as a single-stranded or double-stranded chain, into viral vector, such as retrovirus, adenovirus, adeno-associated virus or the like, or other vector.

(8) An antibody for the polypeptide of the present invention can be produced by the method described in <3> using the polypeptide of the present invention as the antigen.

The polypeptide of the present invention can be detected or determined immunologically using the antibody for the polypeptide of the present invention.

Examples include detection methods, such as ELISA using a microtiter plate, immunocytochemistry by enzyme-labeled antibody method or fluorescent antibody method, Western blotting method and the like.

Examples include sandwich ELISA using two monoclonal antibodies having different epitopes, among antibodies which react with the polypeptide of the present invention in solution, and a radioimmunoassay using an antibody capable of recognizing the polypeptide of the present invention labeled with a radioisotope, such as ¹²⁵I or the like, and the polypeptide of the present invention.

The antibody of the present invention can also be applied to immunohistochemistry using pathologic tissue sections.

The polypeptide of the present invention existing in cells or tissues of a normal person and a patient is immunologically detected or determined using the antibody of the present invention, the amounts between the normal person and patient are compared, and then a difference in expression level is determined to diagnose neurodegenerative diseases (Alzheimer's disease, Parkinson's disease and the like), ischemic diseases, brain damage due to stroke, epilepsy, or various immunological and inflammatory diseases of patients.

(9) Neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis and the like, amyotrophic diseases, such as amyotrophic lateral sclerosis and the like, ischemic diseases, brain damage due to stroke, schizophrenia, depression, epilepsy, or various immunological and inflammatory diseases can be prevented or treated by administering an antibody which inhibits a function of the polypeptide of the present invention (JNK3 scaffold polypeptide or substrate of phosphorylation by JNK3, or binding with JNK3).

The medicament containing the antibody of the present invention can be prepared using a method similar to the preparation method of pharmaceutical preparations of the phosphorylation inhibitor or binding inhibitor of the polypeptide of the present invention described in <4> above, and the thus prepared pharmaceutical preparations can be administered in the same manner as in <4> above.

(10) The phosphorylation inhibitor and binding inhibitor of the present invention and the compound which controls expression of the gene of the polypeptide of the present invention can be used for preventing or treating neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis and the like, amyotrophic diseases, such as amyotrophic lateral sclerosis and the like, ischemic diseases, brain damage due to stroke, schizophrenia, depression, epilepsy, or various immunological and inflammatory diseases.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023]

Fig. 1 is a restriction enzyme map of pcDNA3-S-JSAP1a-Δ1.
 Fig. 2 is a restriction enzyme map of pcDNA3-S-JSAP1a-Δ2.
 Fig. 3 is a restriction enzyme map of pcDNA3-S-JSAP1a-Δ3.
 Fig. 4 is a restriction enzyme map of pcDNA3-S-JSAP1a-Δ4.
 Fig. 5 is a restriction enzyme map of pcDNA3-S-JSAP1a-Δ5.
 Fig. 6 is a restriction enzyme maps of pcDNA3-S-JSAP1a to pcDNA3-S-JSAP1d.
 Fig. 7 is a restriction enzyme map of pcDNA3-S-JSAP3.
 Fig. 8 is a restriction enzyme map of pcDNA3-S-JSAP4.
 Fig. 9 is a restriction enzyme map of pGAD10-JSAP5.
 Fig. 10 is a restriction enzyme map of pcDNA3-His-S-JSAP5.
 Fig. 11 is an electrophoresis pattern showing a result of Northern hybridization for mRNA of the mouse liver, spleen, kidney, brain, heart, lung and testis using a partial sequence of each cDNA of mouse JNK3 and mouse JSAP1a as the probe. A result on β-actin is also shown as an expression control.
 Fig. 12 is an electrophoresis pattern showing a result of test on the binding ability of JSAP1a to JNK1, JNK2, JNK3, ERK2 and p38 analyzed by Western blotting. Lower column shows a result of Western blotting carried out using a cell extract in order to confirm the expression amount of each of Flag-JNK1, 2, 3, ERK2 and p38 in COS-7 cells.
 Fig. 13 is an electrophoresis pattern showing a result of the analysis of JNK3 binding region in JSAP1a.
 Fig. 14 is an electrophoresis pattern showing a result of the phosphorylation of JSAP1a by JNK3.
 Fig. 15 is an electrophoresis pattern showing a result of the analysis of the phosphorylation of JSAP1a and intracellular localization of JNK3. JNK3 was detected by antibody staining. The cell nucleus was detected by Hoechst staining.
 Fig. 16 is an electrophoresis pattern showing a result of Western blotting analysis on the binding ability of JSAP1a to SEK1. Lower two columns show a result of Western blotting carried out using a cell extract in order to confirm the expression level of each Flag-SEK1 and activated SEK1 in COS-7 cells. FL means a full length polypeptide.
 Fig. 17 is an electrophoresis pattern showing a result of Western blotting analysis on the binding ability of JSAP1a to MKK7. Lower column shows a result of Western blotting carried out using a cell extract in order to confirm the expression level of each Flag-MKK7 in COS-7 cells. FL means a full length polypeptide.
 Fig. 18 is an electrophoresis pattern showing a result of Western blotting analysis on the binding ability of JSAP1a to MEK1, MKK6 and MKK7. Lower column shows a result of Western blotting carried out using a cell extract in order to confirm the expression amount of each of Flag-MEK1, MKK6 and MKK7 in COS-7 cells. FL means a full length polypeptide.
 Fig. 19 is an electrophoresis pattern showing a result of Western blotting analysis on the binding ability of JSAP1a to an N-terminal moiety (1st to 640th amino acid residues) of MEKK1. Lower column shows a result of Western blotting carried out using a cell extract in order to confirm the expression amount of the N-terminal moiety of each Flag-MEKK1 in COS-7 cells. FL means a full length polypeptide.
 Fig. 20 is an electrophoresis pattern showing a result of Western blotting analysis on the binding ability of JSAP1a to an N-terminal moiety (1st to 327th amino acid residues) or C-terminal moiety (316th to 648th amino acid residues) of Flag-c-Raf1. Lower column shows a result of Western blotting carried out using a cell extract in order to confirm the expression level of each of Flag-Raf-N and Raf-C in COS-7 cells. FL means a full length polypeptide.
 Fig. 21 shows a result of examination on the effects of Cdc42 and JSAP1a on the JNK3 activity, carried out using a LUC reporter system in which the JNK3 activity in P19 cells is measured as the GAL4-c-Jun transcription activity. The longitudinal axis shows a relative luciferase activity corresponding to the JNK3 activity.
 Fig. 22 shows a result of examination on the effects of ΔRaf1 and JSAP1a on the ERK activity, carried out using a LUC reporter system in which the ERK activity in P19 cells is measured as the GAL4-Elk1 transcription activity. The longitudinal axis shows a relative luciferase activity corresponding to the ERK activity.
 Fig. 23 is an electrophoresis pattern showing a result of Western blotting analysis, carried out using a ΔMEKK1 enzyme solution, a JNK3 enzyme solution and an activated JNK3 enzyme solution obtained by introducing

Δ MEKK1 and JNK3 genes in COS-7 cells singly or simultaneously. "A" shows a result of staining of activated JNK3 with an antibody which recognizes phosphorylated JNK3, and "B" shows a result of staining of JNK3 with anti-Flag antibody. The Δ MEKK1 enzyme solution was applied to lane 1 of the electrophoresis, and the activated JNK3 enzyme solution to lane 2, the JNK3 enzyme solution to lane 3 and a disrupted solution of COS-7 cells without gene introduction to lane 4.

Fig. 24 is an electrophoresis pattern showing a result of Western blotting analysis on the binding ability of JSAP3, 4 and 5 to JNK3. Lower column shows a result of Western blotting carried out using a cell extract in order to confirm the expression amount of each of Flag-JNK3 in COS-7 cells.

Fig. 25 is an electrophoresis pattern showing a result of autoradiography on the binding ability of JSAP3 to ATF2.

Fig. 26 is an electrophoresis pattern showing a result of autoradiography on the expression of 35 S-labeled JSAP4 molecules having various length.

Fig. 27 is an electrophoresis pattern showing a result of autoradiography of the JNK3 binding region of JSAP4. This is a result of the analysis of the binding of 35 S-labeled JSAP4 molecules having various length with GST or GST-JNK3, and it is observed as a band.

Fig. 28 is an electrophoresis pattern showing a result of Western blotting analysis on the binding ability of JSAP4 to JNK1, JNK2 and ERK2. Among the three columns of blotting, the second and third columns show results of Western blotting carried out using cell extracts in order to confirm the expression amounts of JSAP4 and each MAPK (JNK1, JNK2, JNK3 and ERK2), respectively, in COS-7 cells.

Fig. 29 shows a result of Northern hybridization carried out on mRNA of the mouse testis, large intestine, heart, lung, kidney, brain, spleen and liver using a partial sequence of cDNA of JSAP4 as the probe. A result on β -actin is also shown as an expression control.

Fig. 30 shows a result of examination on the effects of MEKK1, TAK1 and JSAP4 on the JNK activity, carried out using a LUC reporter system in which the JNK activity in COS-7 cells is measured as the GAL4-c-Jun transcription activity. The longitudinal axis shows a relative luciferase activity corresponding to the JNK activity.

Fig. 31 shows a result of examination on the effects of Δ Raf1 and JSAP4 on the ERK activity, carried out using a LUC reporter system in which the ERK activity in COS-7 cells is measured as the GAL4-Erk1 transcription activity. The longitudinal axis shows a relative luciferase activity corresponding to the ERK activity.

Description of the Symbols:

[0024]

kb: kilobase pairs

Ap: ampicillin-resistant gene

knt: klonucleotides

BEST MODE FOR CARRYING OUT THE INVENTION

[0025] The present invention is explained based on the examples, but the scope of the present invention is not limited thereto.

[0026] Unless otherwise noted, the known methods described in *Molecular Cloning*, 2nd ed. were used as the gene manipulation techniques in the following examples.

Example 1 Cloning of cDNA encoding a polypeptide having the activity to bind to JNK3

[0027]

(1) Cloning from mouse brain-derived cDNA library

A full length cDNA encoding the mouse JNK3 (*Nature Medicine*, 3: 89 (1997)) was inserted into the *NcoI*-*Bam*HI site of cloning vector pAS2-1 (manufactured by Clontech) containing a sequence encoding GAL4 DNA binding domain (pAS2-1-JNK3), and then introduced into yeast CG-1945 (manufactured by Clontech).

A mouse brain cDNA library in cloning vector pGAD10 (manufactured by Clontech) containing a sequence encoding the GAL4 transcription activation domain was introduced into the above yeast which had been introduced with pAS2-1-JNK3 to obtain yeast transformants.

Strains from which the histidine requirement was removed (capable of growing on a histidine-free medium) or strains having β -galactosidase activity were selected from the yeast transformants (primary positive clones).

Clones having no histidine requirement and having β -galactosidase activity were selected from the resulting primary positive clones (secondary positive clones).

Plasmid derived from pGAD10 was recovered from the resulting clones.

As a result, four kinds of partial length cDNA fragments having different sequences were obtained from the mouse brain cDNA library by a two-hybrid system using the above yeast.

Using these obtained fragments as probes, a λ ZAPII mouse brain cDNA library (manufactured by Clontech) was screened in a known method, and seven kinds of cDNA clones encoding JSAP1a, JSAP1b, JSAP1c, JSAP1d, JSAP3, JSAP4 and JSAP5 as polypeptides capable of binding to JNK3 (JSAP) were obtained.

The cDNA encoding JSAP1a, JSAP1b, JSAP1c, JSAP1d, JSAP3 or JSAP4 was obtained as a full length cDNA, but the cDNA encoding JSAP5 was obtained as a partial length cDNA.

It was found that the cDNA encoding JSAP1a, JSAP1b, JSAP1c, JSAP1d, JSAP3 or JSAP4 contained an open reading frame (hereinafter referred to as "ORF") of 3,918 bp, 3,945 bp, 4,014 bp, 4,011 bp, 1,293 bp or 4,527 bp, respectively, which encoded amino acid residues of 1305 residues, 1314 residues, 1337 residues, 1336 residues or 1508 residues, respectively (SEQ ID NOS:1 to 6).

Also, JSAP1a, JSAP1b, JSAP1c and JSAP1d were splice variants derived from the same gene. That is, a variant in which a nucleotide sequence of 27 bp had been inserted into JSAP1a was JSAP1b, a variant in which nucleotide sequences of 3 bp and 93 bp were inserted was JSAP1c, and a variant in which a nucleotide sequence of 93 bp was inserted was JSAP1d.

Specifically, the insertion site of JSAP1b is a 27 bp DNA sequence moiety encoding 9 amino acid residues from the 201st serine residue to the 209th serine residue of SEQ ID NO:10; the insertion sites of JSAP1c are a 3 bp DNA sequence moiety encoding the 201st serine residue and a 93 bp DNA sequence moiety encoding 31 amino acid residues from the 219th valine residue to the 249th glutamine residue of SEQ ID NO:11; and the insertion site of JSAP1d is a 93 bp DNA sequence moiety encoding 31 amino acid residues from the 218th valine residue to the 248th glutamine residue of SEQ ID NO:12.

JSAP1a coincided with a known sequence (SEQ ID NO:9, 1997 The Molecular Biology Society of Japan (December)).

It was estimated that JSAP3 is a mouse homologue of human C-terminal-binding protein 1 (CtBP) (*EMBO J.*, 17: 5129 (1998)) (SEQ ID NO:13).

In JSAP4 having the amino acid sequence represented by SEQ ID NO:14, a sequence called WD40-repeat was found in the 87th to 121st positions, the 341st to 373rd positions, the 658th to 690th positions and the 700th to 732nd positions of the amino acid sequence.

It is expected that the sequence can be concerned in an interaction (binding) with other protein, and it can be found in a molecule which takes part in the intracellular signal transduction, such as G-protein (*FEBS Lett.*, 307: 131 (1994)). Based on Examples described below, it was revealed that the sequence has an important function on signal transduction in the JNK3 pathway.

The cDNA encoding JSAP5 was a DNA constituted by 734 bp shown in SEQ ID NO:7. It was found that this cDNA does not contain initiation and termination codons and therefore is a partial length. The cDNA encoded 244 amino acid residues (SEQ ID NO:15).

Full length cDNA of JSAP5 (SEQ ID NO:8) was obtained from the λ ZAPII mouse brain cDNA library (manufactured by Clontech) by a known method using a probe prepared based on the information on the cDNA nucleotide sequence of JSAP5. It was found that the cDNA contains an ORF of 1,455 bp and encodes 484 amino acid residues (SEQ ID NO:16, hereinafter referred to as "JSAP5F"). In the following experiment, cDNA of JSAP5 as a partial length cDNA of JSAP5F was used.

(2) Preparation of various polypeptides and vectors expressing the polypeptides for analyzing properties of JSAP

[0028] In order to analyze functions of the resulting polypeptide JSAP capable of binding to JNK3, the following polypeptides and vectors which express the polypeptides were prepared.

1) Preparation of vectors which express fusion polypeptides with thioredoxin-S-tag (hereinafter referred to as "Trx-S") peptide

By a known method, cDNA samples of JNK1, JNK2, JNK3 and SEK1 were obtained from a λ ZAPII mouse brain cDNA library (manufactured by Clontech), and the MEKK1 cDNA from a λ ZAPII mouse spleen cDNA library (manufactured by Clontech).

The c-Raf1 cDNA was obtained from a cDNA library provided by Health Science Research Resources Bank, Japan.

Samples of cDNA of human lymphocyte-derived MKK6, p38, ERK2, c-Jun(1-79) and ATF2 and mouse thymus-derived MEK1, MKK7 and Cdc42 were obtained by using a PCR method.

Each of the resulting cDNA was inserted into the downstream of the Trx-S sequence of pET32a (manufactured by Novagen) to prepare a respective vector which expresses Trx-S-JNK1, Trx-S-JNK2, Trx-S-JNK3, Trx-S-SEK1,

Trx-S-MEKK1, Trx-S-c-Raf1, Trx-S-MKK6, Trx-S-p38, Trx-S-ERK2, Trx-S-c-Jun, Trx-S-ATF2, Trx-S-MEK1, Trx-S-MKK7 or Trx-S-Cdc42.

A DNA encoding the JSAP obtained in the above step (1) or a fragment of the DNA was inserted into each of the restriction enzyme sites described below existing in the downstream of the Trx-S sequence of expression vector pET32a (manufactured by Novagen) to prepare a respective vector which expresses Trx-S-JSAP1a, Trx-S-JSAP1b, Trx-S-JSAP1c, Trx-S-JSAP1d, Trx-S-JSAP3, Trx-S-JSAP4 or Trx-S-JSAP5.

Polypeptide encoded by each DNA inserted and each restriction enzyme site for insertion are shown below.

JSAP1a (115th to 274th amino acid residues):

NcoI-BamHI site

JSAP1a (115th to 504th amino acid residues):

EcoRI site

JSAP1a (268th to 486th amino acid residues):

NcoI site

JSAP1a (486th to 744th amino acid residues):

NcoI-BamHI site

JSAP1a (744th to 1194th amino acid residues):

BamHI site

JSAP1b (115th to 283rd amino acid residues):

NcoI-BamHI site

JSAP1c (115th to 306th amino acid residues):

NcoI-BamHI site

JSAP1d (115th to 305th amino acid residues):

NcoI-BamHI site

JSAP3 (full length):

EcoRI-SalI site

JSAP4 (1042nd to 1331st amino acid residues):

EcoRI-HindIII site

JSAP5 (partial length):

EcoRI site

Also, Trx-S-ATF2 (1st to 107th amino acid residues) and Trx-S-ATF2 (1st to 116th amino acid residues) were prepared by inserting a DNA encoding 1st to 107th amino acid residues of ATF2 and a DNA encoding 1st to 116th amino acid residues of ATF2 into *BamHI-XhoI* and *BamHI-XhoI* sites, respectively, existing in the downstream of the Trx-S sequence of the expression vector pET32a (manufactured by Novagen).

2) Preparation of vectors which express fusion polypeptides with Flag peptide

Each full length cDNA encoding JNK1, JNK2, JNK3, ERK2 or p38 was inserted into the *NotI-BamHI* site existing in the downstream of the Flag sequence of mammal expression vector pFlag-CMV-2 (manufactured by Kodak) to prepare a corresponding vector which expresses Flag-JNK1, Flag-JNK2,

Flag-JNK3, Flag-ERK2 or Flag-p38, respectively.

Also, a DNA encoding each of the following polypeptides was inserted into each of the following restriction enzyme sites existing in the downstream of the Flag sequence of Flag-modified pcDNA3 vector to prepare a respective vector which expresses Flag-SEK1, Flag-MKK6, Flag-MKK7, Flag-MEK1, Flag-MEKK1, Flag-c-Raf1, Flag-Raf-C, Flag-MEKK-N or Flag-TAK1.

Polypeptide encoded by each DNA inserted and each restriction enzyme site for insertion are shown below.

SEK1 (full length):

HindIII-XbaI site

MKK6 (full length):

HindIII-XbaI site

MKK7 (full length):

HindIII-XbaI site

MEK1 (full length):

HindIII-XbaI site

MEKK1 (full length):

BamHI-EcoRV site

c-Raf1 (full length):

EcoRI-XhoI site
 Raf-N (1st to 327th amino acid residues):
EcoRI-EcoRV site
 Raf-C (316th to 648th amino acid residues):
EcoRV-XhoI site
 MEKK-N (1st to 640th amino acid residues):
BamHI-EcoRI site
 TAK1 (full length):
EcoRI-XhoI site

The DNA encoding TAK1 (TGF- β -activated kinase 1) (*Science*, 270: 2008 (1995)) was obtained by a known method from a mouse cell strain BAF-B03 cDNA library.

3) Preparation of vectors which express fusion polypeptides with GST

DNA encoding 1st to 79th amino acid residues of c-Jun into the *BamHI-EcoRI* site of GST fusion protein expression vector pGEX-3X (manufactured by Pharmacia) to prepare GST-c-Jun(1-79) expression vector.

E. coli was transformed using the expression vector by a known method to express GST-c-Jun(1-79).

The thus expressed GST-c-Jun(1-79) was purified using Glutathione Sepharose 4B (manufactured by Pharmacia).

NcoI (blunt ended)-*BamHI* (blunt ended) DNA fragment encoding JNK3 (full length) was inserted into the *BamHI* (blunt ended) site of GST fusion protein expression vector pGEX-2T (manufactured by Pharmacia) to prepare GST-JNK3 expression vector.

E. coli was transformed using the expression vector by a known method to prepare GST-JNK3. The thus expressed GST-JNK3 was purified using Glutathione Sepharose 4B (manufactured by Pharmacia).

4) Preparation of vectors which express fusion polypeptides with S-tag peptide

DNA encoding each of the polypeptides described below or a fragment of the DNA was inserted into each of the restriction enzyme sites described below existing in the downstream of the S-tag sequence of expression vector S-modified pcDNA3 to prepare a respective vector which expresses S-JSAP1a, S-JSAP1a Δ 1, S-JSAP1a Δ 2, S-JSAP1a Δ 3, S-JSAP1a Δ 4, S-JSAP1a Δ 5, S-JSAP1b, S-JSAP1c, S-JSAP1d, S-JSAP3, S-JSAP4, S-JSAP4 (1-754), S-JSAP4 (755-1508), S-JSAP4 (755-1062), S-JSAP4 (1063-1331) or S-JSAP4(1332-1508).

Polypeptide encoded by each DNA inserted and each restriction enzyme site for insertion are shown below.

JSAP1a (full length):
NcoI site
 JSAP1a Δ 1 (1st to 1053rd amino acid residues):
NotI-XhoI site
 JSAP1a Δ 2 (744th to 1305th amino acid residues):
NotI site
 JSAP1a Δ 3 (1054th to 1305th amino acid residues):
BamHI site
 JSAP1a Δ 4 (343rd to 1053rd amino acid residues):
HindIII-XhoI site
 JSAP1a Δ 5 (1st to 343rd amino acid residues):
HindIII site
 JSAP1b (full length):
NotI site
 JSAP1c (full length):
NotI site
 JSAP1d (full length):
NotI site
 JSAP3 (full length):
EcoRI-XhoI/SalI
 JSAP4 (full length):
EcoRI-HindIII
 JSAP4 (1st to 754th amino acid residues):
EcoRI-HindIII
 JSAP4 (755th to 1508th amino acid residues):

EcoRI-HindIII
 JSAP4 (755th to 1062nd amino acid residues):
EcoRI-HindIII
 JSAP4 (1063rd to 1331st amino acid residues):
EcoRI-HindIII
 JSAP4 (1332nd to 1508th amino acid residues):
EcoRI-HindIII

Also, cDNA (partial length) of JSAP5 was inserted into the *EcoRI* site existing in the downstream of the GAL4AD sequence of expression vector pGAD10 (manufactured by Clontech), and cDNA (full length) of JSAP5F into the *EcoRV* (blunt ended)-*HindIII* site existing in downstream of the His-S-tag sequence of expression vector His-S-modified pcDNA3 (Figs. 1 to 10).

5) Preparation of vector which expresses JNK3

Plasmid pGEM-NCO in which the *NcoI* site had been added to pGEM-3Zf(+) (manufactured by Promega) was prepared by digesting pGEM-3Zf(+) with *EcoRI* and then carrying out self-ligation by adding an oligonucleotide linker having a nucleotide sequence of GCCATGC.

JNK3 (full length) was inserted into the *NcoI-BamHI* site of the pGEM-NCO to prepare expression vector (pGEM-JNK3).

6) Preparation of vector which expresses constitutively activated Cdc42

Constitutively activated Cdc42 was prepared by converting the 12th glycine of Cdc42 into valine by point mutation (Cdc42(G12V)). A DNA fragment (*BamHI*-blunt end) encoding the Cdc42(G12V) (full length) was inserted into the *BamHI-EcoRV* site existing in the downstream of S-tag sequence of the expression vector s-modified pcDNA3.

7) Preparation of vector which expresses constitutively activated MEKK1

cDNA encoding ΔMEKK1 (1169th to 1488th amino acid residues; constitutively activated as truncated form of MEKK1) was inserted into the *XbaI* site of pEF-BOS vector (*Nucleic Acids Res.*, 18: 5322 (1990)).

8) 5XGAL4-LUC reporter, GAL4-c-Jun and GAL4-E1k1 expression vectors

All of the 5XGAL4-LUC reporter, GAL4-c-Jun and GAL4-E1k1 expression vectors were purchased from Stratagene and used.

9) RL (*Renilla* luciferase) control vector

The RL control vector was purchased from Promega and used.

10) Preparation of vectors which express fusion polypeptides with Myc-tag peptide

cDNA encoding JSAP4 (full length) or JSAP4 (1063rd to 1331st amino acid residues) was inserted into the *EcoRI-NotI* site existing in the downstream of Myc-tag sequence of the expression vector Myc-modified pcDNA3 to prepare a respective vector which expresses Myc-JSAP4 (full length) or Myc-JSAP4 (1063rd to 1331st).

11) Preparation of vectors which express fusion polypeptides with His-S-tag peptide

DNA encoding each of the polypeptides described below was inserted into each of the restriction enzyme sites described below existing in the downstream of the His-S-tag sequence of expression vector His-S-modified pcDNA3 which encodes the His-S-tag to prepare a respective vector which expresses MAPK-His-S, MAPKKK-His-S, JNK1-His-S, JNK2-His-S, JNK3-His-S, ERK2-His-S or MEKK1-His-S.

The insertion restriction enzyme site of each DNA encoding the full length polypeptide is shown below.

JNK1 (*NotI* (blunt end)-*BamHI* DNA fragment):

EcoRV-BamHI

JNK2 (*NotI* (blunt end)-*BamHI* DNA fragment):

EcoRV-BamHI

JNK3 (*NotI* (blunt end)-*BamHI* (blunt end) DNA fragment):

EcoRV

ERK2 (*BamHI* DNA fragment):

BamHI

MEKK1 (*HindIII* DNA fragment):

HindIII

Example 2 JSAP1a, JSAP1b, JSAP1c and JSAP1d

[0029] Since the results of analysis of JSAP1a, JSAP1b, JSAP1c and JSAP1d described below were identical to one another, the results of JSAP1a are shown in the drawings as the representative example. Hereinafter, JSAP1a, JSAP1b, JSAP1c and JSAP1d are collectively to as "JSAP1".

1) Analysis of the expression of JNK3 and JSAP1 mRNA by Northern hybridization

Northern hybridization was carried out according to the method described in *Proc. Natl. Acad. Sci. USA*, 92: 4972 (1995).

Specifically, each of the liver, spleen, kidney, brain, heart, lung and testis of mouse was analyzed using ³²P-labeled JNK3, JSAP1 and β-actin as the probe.

The results are shown in Fig. 11.

Expression of JNK3 was found specifically in brain. With regard to JSAP1a, JSAP1a mRNA having a size of about 6 kb was found in specifically brain.

2) Analysis of binding specificity and binding region of JSAP1 for various MAPK's

Each of the S-JSAP1a, S-JSAP1b, S-JSAP1c or S-JSAP1d (full length) expression vector or the Flag-JNK1, Flag-JNK2, Flag-JNK3, Flag-ERK2 or Flag-p38 expression vector, prepared in Example 1(2), was transfected to COS-7 cells using TransIT-LT1 (manufactured by Mirus), and then the COS-7 cells were cultured to transiently express the polypeptide derived from the respective introduced vectors.

After culturing for 34 hours, the cells were dissolved in buffer B, S Protein Agarose(manufactured by Novagen) was added thereto, and then S-JSAP1 and a polypeptide capable of binding to S-JSAP1 were precipitated and recovered.

The resulting recovered fraction was separated by SDS-PAGE and transferred on membrane Immobilon-P (manufactured by Millipore).

Western blotting was carried out using the membrane and anti-Flag M5 monoclonal antibody (manufactured by Kodak) as the probe, and the polypeptide capable of binding to the antibody (Flag-JNK3) was visualized using an ECL detection system (manufactured by Amersham) to examine MAPK capable of binding to JSAP1.

The results are shown in Fig. 12. It was found that JSAP1 binds only to JNK3 and does not bind to other MAPK.

The binding region of JSAP1 to JNK3 was analyzed by the following method.

A fusion polypeptide of Trx-S with a partial fragment of JSAP1, each Trx-S-JSAP1 (fragment) was expressed in *E. coli* using the expression vector prepared in Example 1(2) by a known method and then recovered by allowing it to bind to S Protein Agarose.

³⁵S-labeled form of full length JNK3 was prepared by *in vitro* translation using the expression vector pGEM-JNK3 prepared in Example 1(2) by TNT T7 Quick Coupled Transcription/Translation System (manufactured by Promega).

The resulting ³⁵S-labeled JNK3 and Trx-S-JAP1 (fragment) were mixed in buffer A (50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.5% NP-40), and the reaction was carried out at 4°C for 2 hours while stirring the reaction solution by rotating its container tube.

After the reaction, the reactant was washed three times with buffer A, and the resulting precipitate was separated by SDS-PAGE and analyzed by autoradiography.

As a result, it was found that the binding region of JSAP1 to JNK3 is present in a region of the 115th to 274th (JSAP1a), 115th to 283rd (JSAP1b), 115th to 306th (JSAP1c) or 115th to 305th (JSAP1d) amino acid residues.

The results in the case of JSAP1a are shown in Fig. 13. In the case of JSAP1a, the binding region was the 115th to 274th amino acid residues.

3) Phosphorylation of JSAP1 by JNK3 and loss of binding ability of JNK3 by phosphorylation

In the binding region of JSAP1 with JNK3, threonine residues having a possibility of being phosphorylated by proline-directed serine/threonine kinase are present as shown below.

JSAP1a: 234th, 244th and 255th amino acid residues

JSAP1b: 243rd, 253rd and 264th amino acid residues

JSAP1c: 266th, 276th and 287th amino acid residues

JSAP1d: 265th, 275th and 286th amino acid residues

Fusion polypeptides of each JSAP fragment containing the above positions expected to be phosphorylated

with Trx-S, Trx-S-JSAP1a (115th to 274th amino acid residues), Trx-S-JSAP1b (115th to 283rd amino acid residues), Trx-S-JSAP1c (115th to 306th amino acid residues) and Trx-S-JSAP1d (115th to 305th amino acid residues) were prepared according to Example 1(2).

COS-7 cells were transfected using TransIT-LT1 (manufactured by Mirus) with the Flag-JNK3 expression vector and Δ MEKK1 expression vector, or the Flag-JNK3 expression vector alone, prepared in Example 1(2), and then the COS-7 cells were cultured to transiently express the polypeptide derived from each of the introduced vectors.

After culturing for 34 hours, the cells were dissolved in buffer B and then Flag-JNK3 was immunoprecipitated using anti-Flag M5 monoclonal antibody (manufactured by Kodak) immobilized on protein-G-agarose.

Using the resulting JNK3 or activated JNK3 and the Trx-S-JSAP1a (115th to 274th amino acid residues), Trx-S-JSAP1b (115th to 283rd amino acid residues), Trx-S-JSAP1c (115th to 306th amino acid residues) or Trx-S-JSAP1d (115th to 305th amino acid residues) prepared above and bound to S protein agarose, phosphorylation was carried out according to the method described in *Cell*, 76: 1025 (1994) by adding 32 P-labeled ATP ($[\gamma\text{-}^{32}\text{P}]\text{ATP}$). As a positive control of phosphorylation, GST-c-Jun (1st to 79th amino acid residues) was used as the substrate (*EMBO J.*, 15: 2760 (1996)).

The reactant was separated by SDS-PAGE and then analyzed by autoradiography.

The results are shown in Fig. 14. The JSAP1 was efficiently phosphorylated (lane 4 in Fig. 14). Phosphorylation of c-Jun as the positive control was also confirmed (lane 2 of Fig. 14).

Each of the above threonine residues having a possibility of being phosphorylated in JSAP1 was converted into an alanine residue by site-specific mutagenesis using overlapping PCR (*Current Protocols in Molecular Biology* (John Wiley & Sons Inc., New York, 1989)), and the converted polypeptide was expressed and recovered according to the method of Example 1(2).

The resulting polypeptides WT to T-3 are shown below.

(i) Trx-S-JSAP1a (115th to 274th amino acid residue region)

WT: No substitution by an alanine residue
 T-0: Substitution with an alanine residue at the 234th, 244th and 255th positions
 T-1: Substitution with an alanine residue at the 244th and 255th positions
 T-2: Substitution with an alanine residue at the 234th and 255th positions
 T-3: Substitution with an alanine residue at the 234 and 244th positions

(ii) Trx-S-JSAP1b (115th to 283rd amino acid residue region)

WT: No substitution by an alanine residue
 T-0: Substitution with an alanine residue at the 243rd, 253rd and 264th positions
 T-1: Substitution with an alanine residue at the 253rd and 264th positions
 T-2: Substitution with an alanine residue at the 243rd and 264th positions
 T-3: Substitution with an alanine residue at the 243rd and 253rd positions

(iii) Trx-S-JSAP1c (115th to 306th amino acid residue region)

WT: No substitution by an alanine residue
 T-0: Substitution with an alanine residue at the 266th, 276th and 287th positions
 T-1: Substitution with an alanine residue at the 276th and 287th positions
 T-2: Substitution with an alanine residue at the 266th and 287th positions
 T-3: Substitution with an alanine residue at the 266th and 276th positions

(iv) Trx-S-JSAP1d (115th to 305th amino acid residue region)

WT: No substitution by alanine residue
 T-0: Substitution with an alanine residue at the 265th, 275th and 286th positions
 T-1: Substitution with an alanine residue at the 275 and 286th positions
 T-2: Substitution with an alanine residue at the 265 and 286th positions
 T-3: Substitution with an alanine residue at the 265 and 275th positions

Phosphorylation was carried out using the resulting polypeptides.

The results in the case of Trx-S-JSAP1a are shown in Fig. 14.

In all of Trx-S-JSAP1a to d, phosphorylation was not observed only in T-0 in which all of the threonine residues expected to undergo phosphorylation were substituted with alanine (lane 5 in Fig. 14), and WT and all of other alanine substitution products (T-1 to 3) were phosphorylated (lanes 6 to 8 in Fig. 14).

According to the method of Example 2(1), the T-0 which were not phosphorylated in the above, Flag-JNK3 and Δ MEKK1 were expressed in P19 cells which were simultaneously differentiated by retinoic acid, and the cells were stained with anti-Flag M5 monoclonal antibody (manufactured by Kodak).

The same test was carried out using WT which was phosphorylated in the above, Flag-JNK3 and Δ MEKK1, and the cells were stained.

The results in the case of JSAP1a are shown in Fig. 15.

In each case of JSAP1, JNK3 was present only in the cytoplasm and unable to transfer into the nucleus when T-0 was used, but JNK3 was transferred into the nucleus when WT was used.

The above results show that, when JSAP1 is phosphorylated by JNK3, the JNK3 bound to JSAP1 is dissociated from JSAP1 and transferred into the nucleus.

4) Binding of various MAPKK and MAPKKK with JSAP1

COS-7 cells were transfected using TransIT-LT1 (manufactured by Mirus) with three kinds of vectors, the S-JSAP1 (full length) expression vector, the expression vector of Flag-SEK1 (Flag-fusion polypeptide of SEK1 (MKK4) as MAPKK), and the Δ MEKK1 expression vector, or two kinds of vectors, the S-JSAP1 (full length) expression vector and the Flag-SEK1 expression vector, prepared in Example 1(2), and then the COS-7 cells were cultured to transiently express the polypeptide derived from each of the introduced vectors.

After culturing for 34 hours, the cells were dissolved in buffer B and then S-JSAP1 and a polypeptide which binds to S-JSAP1 was immunoprecipitated using S-protein agarose (manufactured by Novagen).

The resulting fraction was separated by SDS-PAGE and transferred on membrane Immobilon-P (manufactured by Millipore), and then Flag-SEK1 was visualized by an ECL detection system (manufactured by Amersham) using anti-Flag M5 monoclonal antibody (manufactured by Kodak) as the probe.

The results are shown in Fig. 16. The binding with JSAP1 was observed when SEB1 was activated by Δ MEKK1 (lanes 2 and 3 in Fig. 16). The activation of SEK1 by Δ MEKK1 was confirmed by Western blotting using a monoclonal antibody which recognizes SEK1 activated by phosphorylation (manufactured by NEB).

Based on the results obtained in the above, the SEK1 binding region in JSAP1 was analyzed in the same manner as the above. That is, various deletion mutants of S-JSAP1 were produced, and binding of each of them to Flag-SEK1 was examined.

The results obtained using deletion mutants derived from JSAP1a are shown in lanes 4 to 8 of Fig. 16. SEK1 was able to bind to the full length FL (1st to 1305th residues), a deletion mutant Δ 2 (having 744th to 1305th residues) and a deletion mutant Δ 3 (having 1054th to 1305th residues) of JSAP1a (lanes 5, 7 and 8 in Fig. 16), but unable to bind to a deletion mutant Δ 1 (having 1st to 1053rd residues) (lane 6 in Fig. 16).

Based on the above, it was found that SEK1 binds to the 1054th to 1305th amino acid residues C-terminal portion of JSAP1a.

Binding of other members of MAPKK to JSAP1a was also analyzed in the same manner as the above.

The results are shown in Figs. 17 and 18.

Similar to the case of SEK1, MKK7 (other MAPKK of the JNK pathway) bound to the 1054th to 1305th residues at the C-terminal portion of JSAP1a (Fig. 17). MEK1 and MKK6 as MAPKK of the ERK or p38 pathway, respectively, also bound to JSAP1a (Fig. 18).

COS-7 cells were transfected using TransIT-LT1 (manufactured by Mirus) with the expression vector of S-JSAP1a (full length) FL, a deletion mutant Δ 1 (having 1st to 1053rd amino acid residues) or a deletion mutant Δ 4 (having 343rd to 1053rd amino acid residues), and the expression vector of Flag-MEKK-N as a fusion polypeptide of an N-terminal sequence polypeptide of an MEKK1, which is MAPKKK (MEKK1-N: 1st to 640th amino acid residues) with Flag-tag, obtained in Example 1(2), and then the COS-7 cells were cultured for transient expression of the polypeptide derived from each of the introduced vectors.

Since the expression of full length MEKK1 in COS-7 cells was considerably low, MEKK1-N as a partial sequence of MEKK1 was used in this experiment.

The results are shown in Fig. 19. The MEKK1-N bound to each of FL, Δ 1 and Δ 4 of JSAP1a. Since it bound to Δ 4, it was considered that MEKK1-N binds to the region of 343rd to 1053rd amino acid residues.

The similar experiment was carried out using a polypeptide having a region other than the N-terminal moiety of MEKK1, and it was confirmed that the polypeptide does not bind to JSAP1a at a region other than the above N-terminal moiety.

Since MEKK1 binds to JSAP1a Δ 1 (1st to 1053rd amino acid residues) having higher affinity than that of the full length JSAP1a (lane 3 in Fig. 19), there is a possibility that the region of 1054th to 1305th residues at the C-terminal of JSAP1a considered in the above to be the binding region of SEK1 has a function of inhibiting the binding

of MEKK1.

In addition, among the MAPK pathway, c-Raf1 as a member of MAPKKK involved in the ERK pathway was examined for its binding to JSAP1.

COS-7 cells were transfected with vector expressing Flag-Raf-N or Flag-Raf-C obtained in Example 1(2), wherein an N-terminal portion region (1st to 327th amino acid residue) or a C-terminal portion region (316th to 648th amino acid residue) of the c-Raf1 is fused with the Flag peptide, and the expression vector of each S-JSAP1 obtained in Example 1(2) using TransIT-LT1 (manufactured by Mirus), and then the COS-7 cells were cultured to transiently express the polypeptide derived from each of the introduced vectors.

In this case, since the expression of full length c-Raf1 in COS-7 cells was considerably low, Raf-N and Raf-C as partial sequences of c-Raf1 were used in this experiment.

The results are shown in Fig. 20. The Raf-C bound to JSAP1 (lane 4 in Fig. 20), but Raf-N did not bind (lane 2 in Fig. 20). Also, the binding affinity of Raf-C was lower than the affinity of MEKK1 (lanes 4 and 6 in Fig. 20).

It was found from the above that JSAP1-binding regions of MAPKKK (MEKK1), MAPK (SEK1 and MKK7) and MAPK (JNK3) related to the JNK3 pathway are different from each other.

In the case of JSAP1a, the binding region for MEKK1 was the 343rd to 1053rd amino acid residue region, the binding region for SEK1 and MKK7 was the 1054th to 1305th residue region, and the binding region for JNK3 was a 115th to 274th residue region.

It was considered that JSAP1 has a leucine-zipper structure. Amino acid sequence numbers of leucine residues constituting the leucine-zipper structure in each JSAP1 are shown below.

JSAP1a: 392, 399, 406, 413, 420, 427

JSAP1b: 401, 408, 415, 422, 429, 436

JSAP1c: 424, 431, 438, 445, 452, 459

JSAP1d: 423, 430, 437, 444, 451, 458

Based on the above, it was considered that each JSAP1 is functioning by existing as a homo or hetero dimer.

5) Analysis of function of JSAP1 as scaffold protein in JNK3 pathway using reporter system

The activation of the JNK3 pathway by over-expression of full length JSAP1 was analyzed.

5XGAL4-LUC reporter expression vector (manufactured by Stratagene), GAL4-c-Jun expression vector (1st to 223rd residues containing a c-Jun activation domain, manufactured by Stratagene) and RL control vector (manufactured by Promega) were introduced into P19 cells differentiated by retinoic acid, originally expressing JSAP1a and JNK3.

Into the P19 cells, full length S-JSAP1a-FL expression vector or S-JSAP1a-Δ5 (1st to 343rd residues) expression vector and/or constitutively activated S-Cdc42 (G12V) expression vector were introduced.

The P19 cells were cultured to transiently express the polypeptide derived from each of the introduced vectors.

After culturing for 24 hours, the luciferase activity was measured, and the GAL4-c-Jun transcription activity, namely JNK3 activity, was calculated. The relative value of the luciferase activity was obtained by correcting it with the activity value of RL luciferase.

The results are shown in Fig. 21.

Cdc42 (G12V) increased the JNK3 activity, and over-expression of JSAP1a increased the JNK3 activity to a similar level of that of Cdc42 (G12V).

When cells in which Cdc42 (G12V) and JSAP1a were simultaneously expressed, the JNK activity was increased additively to the case of single use.

On the other hand, the JNK3 activity was inhibited when JSAP1a-Δ5 (1st to 343rd amino acid residues) and Cdc42 (G12V) were simultaneously expressed.

The influence of over-expression of full length JSAP1 upon the ERK pathway was examined in the same manner as the above.

5XGAL4-LUC reporter expression vector, GAL4-Elk1 (307th to 427th residues containing an Elk1 activation domain) expression vector and RL control vector were introduced into P19 cells differentiated by retinoic acid.

Into the P19 cells were introduced S-JSAP1a-FL (full length JSAP1a) expression vector and/or the expression vector of constitutively activated ΔRaf1 (*Mol. Cell. Biol.*, 9: 639 (1989)) polypeptide with Flag (Flag-ΔRaf1).

The P19 cells were cultured for transient expression of the polypeptide derived from each of the introduced vectors.

The luciferase activity was measured after culturing for 24 hours, and the GAL4-Elk1 transcription activity, namely the ERK activity, was calculated. The relative value of the luciferase activity was obtained by correcting it with the activity value of RL luciferase.

The results are shown in Fig. 22.

It was revealed that over-expression of JSAP1a inhibits the ERK activity.

The JSAP1a bound to all of the MAKPKKK, MAPKK and JNK3 existing in the JNK3 pathway, and it was concluded from the above results that it functions as an important scaffold polypeptide which effectively and specifically activate the JNK3 pathway.

6) Construction of system for screening inhibitor which inhibits phosphorylation of JSAP1 by JNK3

A fusion protein of a partial protein corresponding to the 114th to 274th positions of the amino acid sequence of JSAP1a (containing the JNK3 binding region and three Thr residues to be phosphorylated) with GST was prepared as shown below, to be used as the phosphorylation substrate of JNK3.

[0030] A forward primer having the nucleotide sequence represented by SEQ ID NO:17 and a reverse primer having the nucleotide sequence represented by SEQ ID NO:18 were synthesized as the JSAP1a-specific PCR primers.

[0031] The forward primer has a nucleotide sequence in which an *EcoRV* recognition sequence is introduced into just before a nucleotide sequence corresponding to a region of the 448th to 484th positions in the nucleotide sequence of cDNA encoding the JSAP1a having the nucleotide sequence represented by SEQ ID NO:1.

[0032] The reverse primer has a nucleotide sequence in which a stop codon is introduced into just after a nucleotide sequence corresponding to a complementary sequence of a region of the 909th to 928th positions in the nucleotide sequence of cDNA encoding the JSAP1a having the nucleotide sequence represented by SEQ ID NO:1, and also an *EcoRI* recognition sequence is introduced further just thereafter.

[0033] PCR by KOD DNA polymerase (manufactured by Toyobo) was carried out using these two kinds of primers and, as the template, the expression plasmid pET32a containing full length JSAP1a prepared in Example 1(2) by Thermal cycler 450 (manufactured by Takara).

[0034] The thus amplified JSAP1a cDNA fragment was separated by agarose gel electrophoresis, and the fragment was extracted using Qiaex II DNA Extraction Kit (manufactured by Quiagen).

[0035] In the same manner, a DNA fragment of pBluescript II KS⁻ digested with restriction enzymes *EcoRI* and *EcoRV* was extracted.

[0036] The resulting linear chain DNA fragment of pBluescript II KS⁻ and JSAP1a DNA fragment were connected by carrying out the ligation reaction at 16°C using DNA Ligation Kit (manufactured by Takara) to obtain plasmid pRH1001 in which the JSAP1a DNA fragment is inserted into pBluescript II KS⁻.

[0037] Competent cells of *E. coli* were transformed with the pRH1001, and then the *E. coli* was cultured overnight on LB plate medium (1% tryptone, 0.5% yeast extract, 1% NaCl) to which ampicillin had been added.

[0038] *E. coli* obtained from the thus formed colony was cultured in 2 ml of TB medium (a medium prepared by adding 900 ml of water to 12 g of tryptone, 24 g of yeast extract and 4 ml of glycerol, sterilizing the mixture using an autoclave, cooling it to 60°C and then adding thereto 100 ml of a sterilized potassium phosphate solution (0.17 M KH₂PO₄ and 0.72 M K₂HPO₄)) to which ampicillin had been added, and pRH1001 was extracted from the *E. coli*.

[0039] By digesting the pRH1001 with *EcoRI* and *EcoRV*, and determining nucleotide sequence of the resulting DNA fragment, it was confirmed that it coincides with the nucleotide sequence encoding JSAP1a.

[0040] A DNA fragment of pRH1001 digested with *EcoRI* and *EcoRV* was separated by an agarose gel electrophoresis, and the fragment was extracted using Qiaex II DNA Extraction Kit (manufactured by Quiagen).

[0041] In the same manner, a DNA fragment of pGEX-3X (manufactured by Pharmacia) digested with restriction enzymes *EcoRI* and *SmaI* was extracted.

[0042] The resulting linear chain DNA fragment of pGEX-3X and pRH1001 DNA fragment were connected by carrying out the ligation reaction at 16°C using DNA Ligation Kit (manufactured by Takara) to obtain plasmid pRH1003 in which the JSAP1a DNA fragment is inserted into pBluescript II KS⁻.

[0043] Competent cells of *E. coli* were transformed with the pRH1003, and then the *E. coli* was cultured overnight on LB plate medium to which ampicillin had been added.

[0044] *E. coli* obtained from the thus formed colony was cultured in 2 ml of TB medium to which ampicillin had been added, and pRH1003 was extracted from the *E. coli*.

[0045] The pRH1003 is *E. coli* expression vector having a DNA fragment encoding a GST-JSAP1 fusion protein.

[0046] The above *E. coli* having pRH1003 was pre-cultured at 37°C overnight in 20 ml of TB medium to which ampicillin had been added.

[0047] The resulting culture was added to 440 ml of TB medium to which ampicillin had been added, and cultured at 25°C for 5 hours, and then isopropyl-β-D-thiogalactopyranoside (IPTG) was added to the culture, followed by culturing at 25°C for 15 hours.

[0048] Bacterial cells were recovered from the resulting culture by centrifugation. To the cells, 20 ml of PBS containing 0.1 mg/ml of lysozyme was added and incubated at 4°C for 1 hour.

[0049] After incubation, 200 μl of 10% N-lauroylsarcosine was added thereto and the bacterial cells were disrupted

using a sonicator.

[0050] The thus disrupted bacterial cells were centrifuged at $100,000 \times g$ and 4°C for 1 hour to obtain a cytoplasm fraction.

[0051] The GST-JSAP1 fusion protein was purified from the cytoplasm fraction by the following method using Glutathione Sepharose 4B (manufactured by Pharmacia)

[0052] That is, 5 ml of gel of the Glutathione Sepharose 4B was packed in a column and equilibrated with PBS containing 0.1% N-lauroylsarcosine. The equilibrated Glutathione Sepharose 4B was allowed to adsorb GST-JSAP1 fusion protein and washed with 30 ml of PBS containing N-lauroylsarcosine, and then the GST-JSAP1 fusion protein was eluted with 20 ml of an elution buffer (PBS containing 5 mM reduced form glutathione and 0.1% N-lauroylsarcosine).

The eluate was dialyzed against PBS to obtain a purified GST-JSAP1 fusion protein solution.

[0053] Since the purified GST-JSAP1 fusion protein solution can be preserved at -80°C , it was used by thawing it prior to use.

[0054] ΔMEKK1 , JNK3 and activated JNK3 were prepared and obtained as shown below.

[0055] COS-7 cells were inoculated into a 60 mm plate at a 2×10^5 to 5×10^5 cells/ml DMEM, followed by culturing overnight at 37°C .

[0056] To 300 μl of OPTI-MEM (Gibco BRL, manufactured by Life Technologies), 9 μl of FuGene 6 transfection reagent (manufactured by Hoffmann-La Roche) was added, and to the resulting solution, 0.1 to 0.5 μg of the expression vector inserted with cDNA encoding ΔMEKK1 and/or 4.5 to 4.9 μg of the expression vector inserted with cDNA encoding JNK3, prepared in Example 1(2), were added and incubated at room temperature for 15 minutes.

[0057] The resulting mixture solution was added slowly by three drop portions to the above COS-7 cells and mixed, and then the cells were cultured at 37°C for 30 to 40 hours.

[0058] The cells were recovered using a scraper and washed with 10 ml of PBS.

[0059] To the resulting COS-7 cells, 1 ml of a cell lysis buffer (50 mM HEPES/NaOH (pH 7.6), 150 mM NaCl, 0.3% (V/V) Nonidet P-40, 20 mM MgCl_2 , 1 mM ethyleneglycol bis(β -aminoethyl)-N,N,N',N'-tetraacetic acid (EGTA), 20 mM β -glycerophosphate, 10 mM Na_3VO_4 , 10 mM NaF, 40 $\mu\text{g/ml}$ phenylmethylsulfonyl fluoride (PMSF), 1 $\mu\text{g/ml}$ pepstatin A, 1 $\mu\text{g/ml}$ leupeptin, 1 $\mu\text{g/ml}$ chymostatin, 2 mM dithiothreitol (DTT)) was added to disrupt the cells.

[0060] The resulting cell lysate was centrifuged at $20,000 \times g$ and 4°C to obtain a supernatant. The supernatant of cell lysate derived from cells introduced with an expression vector into which cDNA encoding ΔMEKK1 had been inserted was used as a ΔMEKK1 enzyme solution, the supernatant of cell lysate derived from an expression vector into which cDNA encoding JNK3 had been introduced was used as a JNK3 enzyme solution and the supernatant of cell lysate derived from cells introduced with two kinds of expression vectors into which cDNA encoding ΔMEKK1 and JNK3 had been inserted was used as an activated JNK3 enzyme solution.

[0061] A system for screening an inhibitor which inhibits phosphorylation of JSAP1 by activated JNK3 is described below.

[0062] After the reaction in homogenous solution using activated JNK3, $[\gamma\text{-}^{32}\text{P}]\text{-ATP}$ (74 TBq/mmol, manufactured by New England Nuclear) and GST-JSAP1, the phosphorylation of JSAP1 by activated JNK3 was measured using, as an index, the radioactivity of ^{32}P incorporated into GST-JSAP1.

[0063] A reaction solution for screening (3 μM $[\gamma\text{-}^{32}\text{P}]\text{-ATP}$ (28 kBq/ml), 2.3 μM GST-JSAP1, 20 mM MgCl_2 , 20 mM β -glycerophosphate, 20 mM p-nitrophenyl phosphate, 1 mM Na_3VO_4 , 1 mM NaF, 2 mM DTT, 1% DMSO or a test compound containing 1% DMSO, 2% (V/V) of any one of the three kinds of enzyme solutions prepared in the above (the activated JNK3 enzyme solution is used in the actual screening), 50 mM HEPES/NaOH (pH 7.6)) was dispensed in 100 μl portions into each well of a 96 well plate (manufactured by Corning) incubated at 30°C for 10 to 30 minutes.

[0064] After incubation, 100 μl of ethanol was added to the reaction solution to terminate the reaction and to precipitate GST-JSAP1.

[0065] The precipitate was filtered and adsorbed on UniFilter Plate GF/B (manufactured by Packard) using FilterMate Harvester (manufactured by Packard).

[0066] The plate was washed at least three times with PBS and dried, and then 40 μl of a scintillator Microscint-20 (manufactured by Packard) was added thereto to measure the radioactivity using a detector TopCount-HTS (manufactured by Packard).

[0067] The results are shown in Table 1.

Table 1

Phosphorylation of GST-JSAP1 by COS-7 lysate	
Enzyme solution *1	Phosphorylation activity (pmol/mg/min)
-	23

*1: Enzyme solution used at the time of preparation of the reaction solution for screening

Table 1 (continued)

Phosphorylation of GST-JSAP1 by COS-7 lysate	
Enzyme solution *1	Phosphorylation activity (pmol/mg/min)
Δ MEKK1	28
JNK3	37
Activated JNK3	978

*1: Enzyme solution used at the time of preparation of the reaction solution for screening

[0068] In the above system, GST-JSAP1 was specifically phosphorylated only when the activated JNK3 enzyme solution was used in the reaction solution for screening.

[0069] Accordingly, the above system in which the activated JNK3 enzyme solution was used in the reaction solution for screening has a possibility of being used as a system which can efficiently screen an inhibitor that inhibits phosphorylation of JSAP1 by JNK3. The true phosphorylation activity can be calculated by subtracting the phosphorylation activity in a system which does not use the activated JNK3 enzyme solution.

[0070] The fact that the above activated JNK3 enzyme solution is a solution containing the activated JNK3 was confirmed by the following experiment.

[0071] Using 10 μ g (as the amount of enzyme) of each of the Δ MEKK1 enzyme solution, JNK3 enzyme solution and activated JNK3 enzyme solution prepared above, SDS-PAGE was carried out and each of the electrophoresed proteins was transferred on membrane Hybond-ECL (manufactured by Amersham-Pharmacia Biotech).

[0072] Western blotting was carried out using the membrane, and anti-active JNK polyclonal antibody (manufactured by Promega) and anti-Flag M2 monoclonal antibody (manufactured by Sigma) as probes. Development of color was carried out using a detection buffer (100 mM Tris/HCl (pH 9.5), 100 mM NaCl, 5 mM $MgCl_2$, 0.33 mg/ml nitro blue tetrazolium (NBT), 0.17 mg/ml 5-bromo-4-chloro-3-indolyl phosphate (BCIP), p-toluidine salt). The results are shown in Fig. 23.

[0073] The band of phosphorylated activated JNK3 was observed only in the activated JNK3 enzyme solution (lane 2 in Fig. 23A). The band of JNK3 protein was observed only in the JNK3 enzyme solution (lane 3 in Fig. 23B), and band shift of JNK3 by phosphorylation was observed (lanes 2 and 3 in Fig. 23B).

[0074] Km values of ATP and GST-JSAP1 as the substrates for phosphorylation reaction in the screening system constructed above were calculated according to the method described in *Introduction to Enzyme Kinetics, Biochemical Experimentation*, 21, edited by Masatake Ohnishi, Japan Scientific Societies Press (1987).

[0075] Both of ATP and GST-JSAP1 showed Michaelis-Menten substrate-reaction initial rate curve, and it was found by Lineweaver-Burk plot that Km values of ATP and GST-JSAP1 were 6.3 μ M and 0.48 μ M, respectively.

Example 3 Binding of JSAP3 to JNK3

[0076] COS-7 cells were transfected with the S-JSAP3 (full length) expression vector and the Flag-JNK3 expression vector, prepared in Example 1(2), using TransIT-LT1 (manufactured by Mirus), and then the COS-7 cells were cultured to transiently express the polypeptide derived from each of the introduced vectors.

[0077] After culturing for 34 hours, the cells were dissolved in buffer B, S Protein Agarose was added thereto, and then S-JSAP3 and polypeptide capable of binding to S-JSAP3 were precipitated.

[0078] The resulting fraction was separated by SDS-PAGE and transferred on membrane Immobilon-P (manufactured by Millipore).

[0079] Western blotting was carried out using the membrane and anti-Flag M5 monoclonal antibody (manufactured by Kodak) as the probe, and the polypeptide capable of binding to the antibody (Flag-JNK3) was visualized using ECL detection system (manufactured by Amersham).

[0080] The results are shown in Fig. 24.

[0081] It was confirmed that JSAP3 binds to JNK3 (lane 2 in Fig. 24).

[0082] Binding of JSAP3 to a transcription factor ATF2 and the ATF2 binding region were analyzed by the following method.

[0083] According to the method described in Example 1(2), JSAP3 (full length polypeptide), Trx-S, Trx-S-ATF2 (1st to 107th amino acid residues) and Trx-S-ATF2 (1st to 116th amino acid residues) fusion polypeptide were expressed in *E. coli* and then recovered by allowing them to bind to S Protein Agarose.

[0084] 35 S-labeled form of S-JSAP3 was prepared using the S-JSAP3 (full length) expression vector obtained in Example 1(2) by *in vitro* translation with TNT T7 Quick Coupled Transcription/Translation System (manufactured by Promega).

[0085] The resulting 35 S-labeled form of S-JSAP3 and each of Trx-S, Trx-S-ATF2 (1st to 107th amino acid residues)

and Trx-S-ATF2 (1st to 116th amino acid residues) were mixed in buffer A, and the reaction was carried out at 4°C for 2 hours while stirring the reaction solution by rotating its container tube.

[0086] After the reaction, the reactant was washed three times with buffer A, and the resulting precipitate was separated by SDS-PAGE and analyzed by autoradiography.

[0087] The results are shown in Fig. 25.

[0088] It was found that JSAP3 binds to a region of 108th to 116th amino acid residues of ATF2.

[0089] Among the JSAP3-binding ATF2 region of 108th to 116th residues, a sequence of the 108th to 112th residues completely coincided with an already reported C1BP binding sequence motif PLDLS (*J. Biol. Chem.*, 273: 8549 (1998)).

Example 4 JSAP4

[0090]

1) Binding of JSAP4 to JNK3

COS-7 cells were transfected with the S-JSAP4 (full length) expression vector and the Flag-JNK3 expression vector, prepared in Example 1(2), using TransIT-LT1 (manufactured by Mirus), and then the COS-7 cells were cultured to transiently express the polypeptide derived from each of the introduced vectors.

After culturing for 34 hours, the cells were dissolved in buffer B, S Protein Agarose was added thereto, and then S-JSAP4 and polypeptide capable of binding to S-JSAP4 were precipitated.

The resulting fraction was separated by SDS-PAGE and transferred on membrane Immobilon-P (manufactured by Millipore).

Western blotting was carried out using the membrane and anti-Flag M5 monoclonal antibody (manufactured by Kodak) as the probe, and the polypeptide capable of binding to the antibody (Flag-JNK3) was visualized using ECL detection system (manufactured by Amersham).

The results are shown in Fig. 24.

It was confirmed that JSAP4 binds to JNK3 (lane 4 in Fig. 24).

The JNK3 binding region of JSAP4 was analyzed by the following method.

The GST or GST-JNK3 fusion protein expression vector obtained in Example 1(2) was expressed in *E. coli*, and the protein was adsorbed to Glutathione-Agarose (manufactured by Sigma).

Using the S-JSAP4 expression vector of full length or partial length JSAP4 obtained in Example 1(2), ³⁵S-labeled form of various S-JSAP4 preparations were prepared by *in vitro* translation using TNT T7 Quick Coupled Transcription/Translation System (manufactured by Promega), separated by SDS-PAGE and then analyzed by autoradiography.

The results are shown in Fig. 26.

The resulting ³⁵S-labeled form of each of various S-JSAP4 preparations and the GST or GST-JNK3 fusion protein adsorbed on Glutathione-Agarose were put into a tube containing buffer A and mixed while rotating the tube, and then the mixture was incubated at 4°C for 2 hours.

After incubation, the reactant was washed three times with buffer A, and the protein adsorbed to Glutathione-Agarose was separated by SDS-PAGE and analyzed by autoradiography.

The results are shown in Fig. 27.

It was found that JNK3 binds to a region of the 1063rd to 1331st amino acid residues of JSAP4.

2) Binding of JSAP4 to JNK1 and JNK2

COS-7 cells were co-transfected with the Myc-tag-added Myc-JSAP4 (1063rd to 1331st amino acid residue region; a region which binds to JNK3) expression vector and the His-S-tag-added His-S-JNK1, His-S-JNK2, His-S-JNK3 or His-S-ERK2 expression vector, prepared in Example 1(2), was carried out using FuGene 6 transfection reagent (manufactured by F. Hoffmann-La Roche).

The COS-7 cells were cultured to transiently express the polypeptide derived from each of the introduced vectors.

After culturing for 34 hours, the cells were dissolved in buffer B and immunoprecipitated using S-protein agarose (manufactured by Novagen).

The resulting precipitation fraction was separated by SDS-PAGE and transferred on membrane Immobilon-P (manufactured by Millipore).

Western blotting was carried out using the membrane and anti-Myc monoclonal antibody 9E10 (manufactured by Boehringer Mannheim) as the probe, and the Myc-JSAP4 capable of binding to the antibody was visualized using ECL detection system (manufactured by Amersham). Also, the expression of Myc-JSAP4 in respective co-transfected cells was confirmed by Western blotting using anti-Myc monoclonal antibody 9E10 (manufactured by Boehringer Mannheim), and the expression of His-S-JNK1, His-S-JNK2, His-S-JNK3 and His-S-ERK2 was con-

firming by Western blotting using anti-His polyclonal antibody (manufactured by Santa Cruz) after the cells were lysed.

The results are shown in Fig. 28.

It was revealed that JSAP4 binds to JNK1, JNK2 and JNK3, whereas it does not bind to ERK2.

3) Expression analysis of JSAP4 mRNA by Northern hybridization

Northern hybridization was carried out according to the method described in *Proc. Natl. Acad. Sci. USA*, 92: 4972 (1995).

Specifically, each of mouse testis, large intestine, heart, lung, kidney, brain, spleen and liver was analyzed using ³²P-labeled JSAP4 and β -actin cDNA probes.

The results are shown in Fig. 29.

Expression of JSAP4 was found slightly in the testis, heart and kidney, and particularly, its most abundant expression was found in the brain.

Since a sequence called WD40-repeat was found in JSAP4, it was expected that the sequence could be concerned in an interaction (binding) with other protein, and it was expected that it would take part in the JNK3 pathway on its signal transduction (*FEBS Lett.*, 307: 131 (1994)).

4) Function analysis of JSAP4 in JNK3 pathway using reporter system

Full length JSAP4 was over-expressed, and influence thereof on the JNK3 pathway was analyzed by the following method.

5XGAL4-LUC reporter expression vector (manufactured by Stratagene), GAL4-c-Jun expression vector (1st to 223rd residues containing a c-Jun activation domain, manufactured by Stratagene) and RL control vector (manufactured by Promega) were introduced into COS-7 cells using FuGENE6 transfection reagent (manufactured by F. Hoffmann-La Roche).

At least one of the Myc-JSAP4-FL expression vector, His-S-MEKK1 expression vector and Flag-TAK1 expression vector prepared in Example 1(2) was introduced into the COS-7 cells using the FuGENE6 transfection reagent.

The COS-7 cells were cultured to transiently express the polypeptide derived from each of the introduced vectors.

The luciferase activity was measured after culturing for 34 hours, and the GAL4-c-Jun transcription activity, namely JNK3 activity, was calculated. The relative value of the luciferase activity was obtained by correcting it with the activity value of RL luciferase.

The results are shown in Fig. 30.

Increase in the JNK activity was slight by the JSAP4 *per se*, whereas the MEKK1 and TAK1 as members of MAPKKK increased the JNK activity 3 times and 3.2 times, respectively. However, the activation of JNK by MEKK1 and TAK1 was further 2.7 times and 3 times increased, respectively, by the over-expression of JSAP4.

Influence of the over-expression of full length JSAP4 on the ERK pathway was examined in the same manner as the above.

5XGAL4-LUC reporter expression vector, GAL4-Elk1 (307th to 427th residues containing an Elk1 activation domain) expression vector and RL control vector were introduced into COS-7 cells using the FuGENE6 transfection reagent.

The Myc-JSAP4-FL expression vector and/or the expression vector of continuously activated Δ Raf1 (*Mol. Cell. Biol.*, 9: 639 (1989)) polypeptide with Flag (Flag- Δ Raf1) prepared in Example 1(2) were introduced into the COS-7 cells using the FuGENE6 transfection reagent.

The COS-7 cells were cultured to transiently express the polypeptide derived from each of the introduced vectors.

The luciferase activity was measured after culturing for 34 hours, and the GAL4-Elk1 transcription activity, namely ERK activity, was calculated. The relative value of the luciferase activity was obtained by correcting it with the activity value of RL luciferase.

The results are shown in Fig. 31.

The over-expression of JSAP4 had no influence on the activity of the ERK pathway. Also, it had no influence on the activation of ERK by the activated Δ Raf1.

Based on the results of 1) and 2) of Example 4, it was concluded that JSAP4 binds to JNK1, JNK2 and JNK3 and has a function of specifically increasing efficiency of the activation of the JNK pathway.

Example 5 Binding of JSAP5 to JNK3

[0091] Transfection of COS-7 cells with the S-JSAP5 (partial length) expression vector and Flag-JNK3 expression

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vector prepared in Example 1 (2) was carried out using TransIT-LT1 (manufactured by Mirus), and then the COS-7 cells were cultured for transient expression of the polypeptide derived from each of the introduced vectors.

[0092] After 34 hours of the culturing, the cells were dissolved in buffer B, S Protein Agarose was added thereto, and then S-JSAP5 and polypeptide capable of binding to S-JSAP5 were precipitated.

5 [0093] The resulting fraction was separated by SDS-PAGE and transferred on membrane Immobilon-P (manufactured by Millipore).

[0094] Western blotting was carried out using the membrane and anti-Flag M5 monoclonal antibody (manufactured by Kodak) as the probe, and the polypeptide capable of binding to the antibody (Flag-JNK3) was visualized using ECL detection system (manufactured by Amersham).

10 [0095] The results are shown in Fig. 24.

[0096] It was confirmed that JSAP5 binds to JNK3 (lane 6 of Fig. 24).

INDUSTRIAL APPLICABILITY

15 [0097] Neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis and the like, amyotrophic diseases, such as amyotrophic lateral sclerosis and the like, ischemic diseases, brain damage due to stroke, schizophrenia, depression, epilepsy, and various immunological and inflammatory diseases can be prevented and treated using the DNA of the novel polypeptide having a JNK3 binding activity obtained in the present invention.

20

FREE TEXT OF SEQUENCE LISTINGS:

[0098]

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SEQ ID NO:18-Explanation of artificial sequence: Synthetic DNA

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45

50

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SEQUENCE LISTING

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 Met Met Glu
 1
 15 atc cag atg gac gag gga gga ggt gtg gtg gtg tac caa gac gac tac 163
 20 Ile Gln Met Asp Glu Gly Gly Gly Val Val Val Tyr Gln Asp Asp Tyr
 5 10 15
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 Cys Ser Gly Ser Val Met Ser Glu Arg Val Ser Gly Leu Ala Gly Ser
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	70	75	80	
5				
	cgc gag gac aac gag cag ctg ctc acg caa tac gag cgc gag aag gcg	403		
10	Arg Glu Asp Asn Glu Gln Leu Leu Thr Gln Tyr Glu Arg Glu Lys Ala			
	85	90	95	
15				
	ctg cgc aaa cag gcc gag gag aaa ttc atc gaa ttt gaa gat gcc ttg	451		
	Leu Arg Lys Gln Ala Glu Glu Lys Phe Ile Glu Phe Glu Asp Ala Leu			
20	100	105	110	115
25				
	gaa caa gag aag aaa gaa ctc cag atc cag gta gaa cat tat gag ttt	499		
	Glu Gln Glu Lys Lys Glu Leu Gln Ile Gln Val Glu His Tyr Glu Phe			
	120	125	130	
30				
	cag aca cgc cag ctg gag cta aag gcc aaa aac tat gca gat cag att	547		
35	Gln Thr Arg Gln Leu Glu Leu Lys Ala Lys Asn Tyr Ala Asp Gln Ile			
	135	140	145	
40				
	tcc cga ctg gag gaa cga gaa tcg gag atg aag aag gaa tac aat gcc	595		
	Ser Arg Leu Glu Glu Arg Glu Ser Glu Met Lys Lys Glu Tyr Asn Ala			
45	150	155	160	
50				
	ctg cac cag cgg cac aca gag atg atc cag acc tat gtg gaa cac att	643		
	Leu His Gln Arg His Thr Glu Met Ile Gln Thr Tyr Val Glu His Ile			
	165	170	175	
55				

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5 gaa aga tcc aag atg cag caa gtt ggg ggt agc ggc caa aca gaa agc 691
 Glu Arg Ser Lys Met Gln Gln Val Gly Gly Ser Gly Gln Thr Glu Ser
 180 185 190 195

10 agc ctg ccc ggg cgg agg aag gag cgt ccc acc tct ctg aat gtc ttc 739
 15 Ser Leu Pro Gly Arg Arg Lys Glu Arg Pro Thr Ser Leu Asn Val Phe
 200 205 210

20 ccc ctg gct gat ggc atg tgt cca aac gat gag atg tct gag tca ggc 787
 Pro Leu Ala Asp Gly Met Cys Pro Asn Asp Glu Met Ser Glu Ser Gly
 25 215 220 225

30 cag tcc tca gca gct gca aca ccc agt acc aca ggt acc aag tcc aac 835
 Gln Ser Ser Ala Ala Ala Thr Pro Ser Thr Thr Gly Thr Lys Ser Asn
 230 235 240

35 aca ccc acg tcc tcc gtg ccc tca gca gca gtc acg cca ctc aac gag 883
 Thr Pro Thr Ser Ser Val Pro Ser Ala Ala Val Thr Pro Leu Asn Glu
 40 245 250 255

45 agc cta cag ccc ctg ggg gac tat gtc agt gtc aca aag aac aac aag 931
 Ser Leu Gln Pro Leu Gly Asp Tyr Val Ser Val Thr Lys Asn Asn Lys
 260 265 270 275

50 cag gcc cga gag aag cgc aat agc cgt aac atg gag gtc cag gtc acc 979
 55 Gln Ala Arg Glu Lys Arg Asn Ser Arg Asn Met Glu Val Gln Val Thr

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	280	285	290	
5				
	caa gag atg cgg aac gtc agt atc ggc atg ggc agc agt gac gag tgg	1027		
10	Gln Glu Met Arg Asn Val Ser Ile Gly Met Gly Ser Ser Asp Glu Trp			
	295	300	305	
15				
	tcc gat gtt cag gac att atc gac tcc acc cca gag ctg gat gtg tgt	1075		
	Ser Asp Val Gln Asp Ile Ile Asp Ser Thr Pro Glu Leu Asp Val Cys			
20	310	315	320	
25				
	cct gaa acc cgt ctg gag cgc aca gga agc agc cca acc cag gga att	1123		
	Pro Glu Thr Arg Leu Glu Arg Thr Gly Ser Ser Pro Thr Gln Gly Ile			
	325	330	335	
30				
	gta aac aaa gct ttt gga atc aac act gac tcc ttg tat cac gaa ctc	1171		
35	Val Asn Lys Ala Phe Gly Ile Asn Thr Asp Ser Leu Tyr His Glu Leu			
	340	345	350	355
40				
	tcc acg gcg gga tct gag gtc atc ggg gat gtg gac gag gga gct gat	1219		
	Ser Thr Ala Gly Ser Glu Val Ile Gly Asp Val Asp Glu Gly Ala Asp			
45	360	365	370	
50				
	ctc cta ggg gag ttt tca gtg cgc gat gat ttt ttt gga atg ggc aaa	1267		
	Leu Leu Gly Glu Phe Ser Val Arg Asp Asp Phe Phe Gly Met Gly Lys			
	375	380	385	

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5 gaa gtg ggg aac ctg ctg ctg gag aac tca cag ctt cta gag aca aaa 1315
 Glu Val Gly Asn Leu Leu Leu Glu Asn Ser Gln Leu Leu Glu Thr Lys
 390 395 400

10

15 aat gct tta aat gta gtg aag aat gac ctc att gct aag gtt gac caa 1363
 Asn Ala Leu Asn Val Val Lys Asn Asp Leu Ile Ala Lys Val Asp Gln
 405 410 415

20

25 ctg tca gga gaa cag gag gtc ctg aag ggt gag ctg gaa gca gcc aag 1411
 Leu Ser Gly Glu Gln Glu Val Leu Lys Gly Glu Leu Glu Ala Ala Lys
 420 425 430 435

30

35 caa gcg aaa gtc aag ctg gag aac cga atc aaa gag ctt gaa gaa gaa 1459
 Gln Ala Lys Val Lys Leu Glu Asn Arg Ile Lys Glu Leu Glu Glu Glu
 440 445 450

40

45 ctg aag aga gtc aag tca gag gca gta act gcc cgc cgt gag ccc aga 1507
 Leu Lys Arg Val Lys Ser Glu Ala Val Thr Ala Arg Arg Glu Pro Arg
 455 460 465

50

55 gaa gag gtg gag gat gta agc agc tat ctc tgt aca gaa ttg gac aaa 1555
 Glu Glu Val Glu Asp Val Ser Ser Tyr Leu Cys Thr Glu Leu Asp Lys
 470 475 480

atc ccc atg gcc cag cgc cga cgc ttc aca cgg gtg gag atg gcc cga 1603
 Ile Pro Met Ala Gln Arg Arg Arg Phe Thr Arg Val Glu Met Ala Arg

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	485	490	495	
5				
	gtg ctc atg gaa cgc aac cag tac aag gaa cgc ctc atg gag ctg cag	1651		
10	Val Leu Met Glu Arg Asn Gln Tyr Lys Glu Arg Leu Met Glu Leu Gln			
	500	505	510	515
15				
	gag gct gtg agg tgg act gaa atg atc aga gca tca agg gaa cac cca	1699		
	Glu Ala Val Arg Trp Thr Glu Met Ile Arg Ala Ser Arg Glu His Pro			
20		520	525	530
25				
	tct gtc cag gag aag aag aag tcc acc atc tgg cag ttc ttt agt cgc	1747		
	Ser Val Gln Glu Lys Lys Lys Ser Thr Ile Trp Gln Phe Phe Ser Arg			
		535	540	545
30				
	ctc ttc agc tcc tca tct agc ccc cct ccg gcc aaa cga tcc tac cca	1795		
	Leu Phe Ser Ser Ser Ser Ser Pro Pro Pro Ala Lys Arg Ser Tyr Pro			
35		550	555	560
40				
	tct gtg aac att cac tac aag tca ccc act gca gct ggc ttt agc cag	1843		
	Ser Val Asn Ile His Tyr Lys Ser Pro Thr Ala Ala Gly Phe Ser Gln			
		565	570	575
45				
	cgt cgc agc cat gct ttg tgc cag atc tca gcc ggc agc agg ccc ctg	1891		
50	Arg Arg Ser His Ala Leu Cys Gln Ile Ser Ala Gly Ser Arg Pro Leu			
	580	585	590	595
55				

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5 gag ttc ttc cct gat gat gac tgc acc tct tct gcc cgg cgg gag cag 1939
 Glu Phe Phe Pro Asp Asp Asp Cys Thr Ser Ser Ala Arg Arg Glu Gln
 600 605 610

10 aag cgg gag cag tac cgc cag gtt cgt gaa cac gtg cgc aat gat gac 1987
 Lys Arg Glu Gln Tyr Arg Gln Val Arg Glu His Val Arg Asn Asp Asp
 15 615 620 625

20 ggg agg ctg cag gcc tgt ggg tgg agc ctg cct gcc aag tac aag cag 2035
 Gly Arg Leu Gln Ala Cys Gly Trp Ser Leu Pro Ala Lys Tyr Lys Gln
 25 630 635 640

30 ctg agc ccc aat gga ggc cag gaa gac acc cgg atg aaa aat gtg cct 2083
 Leu Ser Pro Asn Gly Gly Gln Glu Asp Thr Arg Met Lys Asn Val Pro
 645 650 655

35 gtc cct gtg tac tgt cgc cct ctg gtg gag aag gac cct tcg aca aag 2131
 Val Pro Val Tyr Cys Arg Pro Leu Val Glu Lys Asp Pro Ser Thr Lys
 40 660 665 670 675

45 ctg tgg tgt gct gct ggt gtc aac ctg agt ggg tgg aag cca cat gaa 2179
 Leu Trp Cys Ala Ala Gly Val Asn Leu Ser Gly Trp Lys Pro His Glu
 680 685 690

50 gag gac tct agc aat gga ccc aag cct gta cca ggt cga gac cct ctg 2227
 Glu Asp Ser Ser Asn Gly Pro Lys Pro Val Pro Gly Arg Asp Pro Leu
 55

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	695	700	705	
5				
	acc tgt gac cgg gaa gga gaa ggc gaa ccc aag agc aca cac cca tca	2275		
10	Thr Cys Asp Arg Glu Gly Glu Gly Glu Pro Lys Ser Thr His Pro Ser			
	710	715	720	
15				
	cct gag aag aag aag gca aag gaa acc cct gag gca gat gct acc tcc	2323		
	Pro Glu Lys Lys Lys Ala Lys Glu Thr Pro Glu Ala Asp Ala Thr Ser			
20	725	730	735	
25				
	agt cgg gta tgg atc ctc acc agc acc ctg aca acc agc aag gtg gtg	2371		
	Ser Arg Val Trp Ile Leu Thr Ser Thr Leu Thr Thr Ser Lys Val Val			
	740	745	750	755
30				
	atc att gat gcc aac cag cca ggc aca att gtg gat cag ttc aca gtc	2419		
35	Ile Ile Asp Ala Asn Gln Pro Gly Thr Ile Val Asp Gln Phe Thr Val			
	760	765	770	
40				
	tgc aat gcc cac gtc ctg tgt atc tcc agc att cct gcg gcc agt gac	2467		
	Cys Asn Ala His Val Leu Cys Ile Ser Ser Ile Pro Ala Ala Ser Asp			
45	775	780	785	
50				
	agt gac tat ccc cct ggg gag atg ttc cta gac agt gat gtg aac cct	2515		
	Ser Asp Tyr Pro Pro Gly Glu Met Phe Leu Asp Ser Asp Val Asn Pro			
	790	795	800	
55				

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5	gaa gat tca ggt gct gat ggt gtg ctg gct ggc atc acc ctg gtg ggg	2563
	Glu Asp Ser Gly Ala Asp Gly Val Leu Ala Gly Ile Thr Leu Val Gly	
10	805 810 815	
15	tgt gct acc cgc tgc aat gtt cca cgt agc aac tgt tcc tca cga gga	2611
	Cys Ala Thr Arg Cys Asn Val Pro Arg Ser Asn Cys Ser Ser Arg Gly	
	820 825 830 835	
20	gac acc cca gta ctg gac aag ggg cag ggg gat gtg gcg acc act gcc	2659
	Asp Thr Pro Val Leu Asp Lys Gly Gln Gly Asp Val Ala Thr Thr Ala	
25	840 845 850	
30	aat ggg aag gtc aac ccg tcc caa tcc aca gaa gaa gcc aca gaa gcc	2707
	Asn Gly Lys Val Asn Pro Ser Gln Ser Thr Glu Glu Ala Thr Glu Ala	
	855 860 865	
35	aca gag gtg cca gac cct ggt ccc agc gag tca gaa gca acg aca gtc	2755
	Thr Glu Val Pro Asp Pro Gly Pro Ser Glu Ser Glu Ala Thr Thr Val	
40	870 875 880	
45	cgg ccc ggg cct ctc aca gag cat gtc ttt act gac cca gca ccc acc	2803
	Arg Pro Gly Pro Leu Thr Glu His Val Phe Thr Asp Pro Ala Pro Thr	
50	885 890 895	
55	cca tcc tcc agc acc cag cct gcc agt gag aat ggg tca gaa tcc aat	2851
	Pro Ser Ser Ser Thr Gln Pro Ala Ser Glu Asn Gly Ser Glu Ser Asn	

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5	900	905	910	915	
	ggc acc att gta cag cct cag gtg gag ccc agt ggg gaa ctc tca aca				2899
10	Gly Thr Ile Val Gln Pro Gln Val Glu Pro Ser Gly Glu Leu Ser Thr				
	920		925	930	
15	aca acc agt agc gct gca ccc act atg tgg cta gga gcc cag aat ggc				2947
	Thr Thr Ser Ser Ala Ala Pro Thr Met Trp Leu Gly Ala Gln Asn Gly				
20	935		940	945	
25	tgg ctc tat gtg cat tca gcg gta gcc aac tgg aag aag tgt ctg cac				2995
	Trp Leu Tyr Val His Ser Ala Val Ala Asn Trp Lys Lys Cys Leu His				
	950		955	960	
30					
	tcc atc aag cta aaa gac tct gtg ctg agc ctg gtg cat gtc aaa ggc				3043
35	Ser Ile Lys Leu Lys Asp Ser Val Leu Ser Leu Val His Val Lys Gly				
	965		970	975	
40	cga gtg ctg gta gct ctt gca gat ggg acc ctg gct atc ttc cat cgt				3091
	Arg Val Leu Val Ala Leu Ala Asp Gly Thr Leu Ala Ile Phe His Arg				
45	980	985	990	995	
50	gga gag gat ggc cag tgg gac ctg agc aac tac cac cta atg gac ctg				3139
	Gly Glu Asp Gly Gln Trp Asp Leu Ser Asn Tyr His Leu Met Asp Leu				
	1000		1005	1010	
55					

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5 ggc cac cca cac cac tcc atc cgc tgc atg gct gtt gtg aat gac cga 3187
 Gly His Pro His His Ser Ile Arg Cys Met Ala Val Val Asn Asp Arg
 1015 1020 1025

10

15 gtt tgg tgt ggc tac aag aac aag gtg cat gtt atc cag ccc aag aca 3235
 Val Trp Cys Gly Tyr Lys Asn Lys Val His Val Ile Gln Pro Lys Thr
 1030 1035 1040

20

25 atg cag att gag aaa tca ttt gat gcc cac cca agg cgg gaa agc cag 3283
 Met Gln Ile Glu Lys Ser Phe Asp Ala His Pro Arg Arg Glu Ser Gln
 1045 1050 1055

30

35 gta cgt cag ctg gcc tgg atc ggt gat gga gtg tgg gtc tct att cgc 3331
 Val Arg Gln Leu Ala Trp Ile Gly Asp Gly Val Trp Val Ser Ile Arg
 1060 1065 1070 1075

40

45 ttg gat tct acc ctt cgg etc tac cat gct cac acc cac cag cac ctg 3379
 Leu Asp Ser Thr Leu Arg Leu Tyr His Ala His Thr His Gln His Leu
 1080 1085 1090

50

55 cag gat gtg gac att gag ccc tat gtt agc aag atg cta gga acc ggc 3427
 Gln Asp Val Asp Ile Glu Pro Tyr Val Ser Lys Met Leu Gly Thr Gly
 1095 1100 1105

55

aag ctg ggc ttc tcc ttc gtg cgc atc aca gcc tta etc att gca ggc 3475
 Lys Leu Gly Phe Ser Phe Val Arg Ile Thr Ala Leu Leu Ile Ala Gly

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	1110	1115	1120	
5				
	aac cgt ctg tgg gtg ggc act ggc aat ggg gtt gtc atc tcc atc ccc			3523
10	Asn Arg Leu Trp Val Gly Thr Gly Asn Gly Val Val Ile Ser Ile Pro			
	1125	1130	1135	
15				
	ttg act gag act gtg gtc ctg cat cga ggc cag ctc cta ggg ctc cga			3571
	Leu Thr Glu Thr Val Val Leu His Arg Gly Gln Leu Leu Gly Leu Arg			
20	1140	1145	1150	1155
25				
	gcc aac aag aca tcc cca aca tct ggg gag ggg acc cgc cca ggg ggc			3619
	Ala Asn Lys Thr Ser Pro Thr Ser Gly Glu Gly Thr Arg Pro Gly Gly			
	1160	1165	1170	
30				
	atc atc cat gtg tat ggg gac gac agc agt gac aag gcc gcc agt agt			3667
	Ile Ile His Val Tyr Gly Asp Asp Ser Ser Asp Lys Ala Ala Ser Ser			
35	1175	1180	1185	
40				
	ttc atc ccc tac tgc tcc atg gca cag gct cag ctt tgc ttc cat ggg			3715
	Phe Ile Pro Tyr Cys Ser Met Ala Gln Ala Gln Leu Cys Phe His Gly			
	1190	1195	1200	
45				
	cac cgt gat gct gtc aaa ttc ttt gtc tct gtg cca gga aat gtg ctg			3763
50	His Arg Asp Ala Val Lys Phe Phe Val Ser Val Pro Gly Asn Val Leu			
	1205	1210	1215	
55				

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5 gcc act ctc aat ggc agt gtg cta gac agc cca tca gag ggc cct ggg 3811
 Ala Thr Leu Asn Gly Ser Val Leu Asp Ser Pro Ser Glu Gly Pro Gly
 1220 1225 1230 1235

10 cct gct gca ccc gct gca gat gct gag ggc cag aag ttg aag aat gca 3859
 Pro Ala Ala Pro Ala Ala Asp Ala Glu Gly Gln Lys Leu Lys Asn Ala
 1240 1245 1250

20 ctg gtg ctg agt ggt ggt gaa ggt tac att gac ttc cgt atc gga gac 3907
 Leu Val Leu Ser Gly Gly Glu Gly Tyr Ile Asp Phe Arg Ile Gly Asp
 25 1255 1260 1265

30 gga gag gat gat gaa act gag gaa tgt gcc ggg gac gtg aac cag aca 3955
 Gly Glu Asp Asp Glu Thr Glu Glu Cys Ala Gly Asp Val Asn Gln Thr
 1270 1275 1280

35 aag ccc tcg ttg tcc aag gct gag cgc agc cac atc atc gtg tgg cag 4003
 Lys Pro Ser Leu Ser Lys Ala Glu Arg Ser His Ile Ile Val Trp Gln
 40 1285 1290 1295

45 gtg tcc tac acc cct gag tgagaccctg tctacctga tgccaactgt 4051
 Val Ser Tyr Thr Pro Glu
 50 1300 1305

55 acataggacc ctacctgcct gcctccccgc ctgttcctg gggcagccag gtctgtccat 4111

5 ccccttttaa cctctcaact tgcagctttt gcctgaggtc cagcccctag ctgttagaga 4171

88 4173

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40 tcggggccccg gaacgagccg cgctggcggc ggcgggcgta gccgcg atg atg gag 115
Met Met Glu
1

45 atc cag atg gac gag gga gga ggt gtg gtg gtg tac caa gac gac tac 163
Ile Gln Met Asp Glu Gly Gly Gly Val Val Val Tyr Gln Asp Asp Tyr

50 5 10 15

55 tgc tcg ggc tcg gtc atg tcg gag cgt gtg tcg ggc ctg gcg ggc tcc 211

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5 Cys Ser Gly Ser Val Met Ser Glu Arg Val Ser Gly Leu Ala Gly Ser
 20 25 30 35
 10 atc tac cgc gag ttc gag cgc ctc att cac tgc tat gac gag gag gtg 259
 Ile Tyr Arg Glu Phe Glu Arg Leu Ile His Cys Tyr Asp Glu Glu Val
 40 45 50
 15 gtc aag gag ctc atg ccg ctg gtg gtg aac gtg ctg gag aac ctt gac 307
 20 Val Lys Glu Leu Met Pro Leu Val Val Asn Val Leu Glu Asn Leu Asp
 55 60 65
 25 tcg gtg ctg agc gag aac cag gag cac gag gtg gag ctg gag ctc cta 355
 Ser Val Leu Ser Glu Asn Gln Glu His Glu Val Glu Leu Glu Leu Leu
 30 70 75 80
 35 cgc gag gac aac gag cag ctg ctc acg caa tac gag cgc gag aag gcg 403
 Arg Glu Asp Asn Glu Gln Leu Leu Thr Gln Tyr Glu Arg Glu Lys Ala
 85 90 95
 40 ctg cgc aaa cag gcc gag gag aaa ttc atc gaa ttt gaa gat gcc ttg 451
 45 Leu Arg Lys Gln Ala Glu Glu Lys Phe Ile Glu Phe Glu Asp Ala Leu
 100 105 110 115
 50 gaa caa gag aag aaa gaa ctc cag atc cag gta gaa cat tat gag ttt 499
 Glu Gln Glu Lys Lys Glu Leu Gln Ile Gln Val Glu His Tyr Glu Phe
 55 120 125 130

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5
cag aca cgc cag ctg gag cta aag gcc aaa aac tat gca gat cag att 547
Gln Thr Arg Gln Leu Glu Leu Lys Ala Lys Asn Tyr Ala Asp Gln Ile

10
135 140 145

15
tcc cga ctg gag gaa cga gaa tcg gag atg aag aag gaa tac aat gcc 595
Ser Arg Leu Glu Glu Arg Glu Ser Glu Met Lys Lys Glu Tyr Asn Ala

20
150 155 160

25
ctg cac cag cgg cac aca gag atg atc cag acc tat gtg gaa cac att 643
Leu His Gln Arg His Thr Glu Met Ile Gln Thr Tyr Val Glu His Ile

30
165 170 175

35
gaa aga tcc aag atg cag caa gtt ggg ggt agc ggc caa aca gaa agc 691
Glu Arg Ser Lys Met Gln Gln Val Gly Gly Ser Gly Gln Thr Glu Ser

40
180 185 190 195

45
agc ctg ccc ggg cgg agt cct cgc cag tcg tgg agg aaa agc agg aag 739
Ser Leu Pro Gly Arg Ser Pro Arg Gln Ser Trp Arg Lys Ser Arg Lys

50
200 205 210

55
gag cgt ccc acc tct ctg aat gtc ttc ccc ctg gct gat ggc atg tgt 787
Glu Arg Pro Thr Ser Leu Asn Val Phe Pro Leu Ala Asp Gly Met Cys

215 220 225

cca aac gat gag atg tct gag tca ggc cag tcc tca gca gct gca aca 835

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5 Pro Asn Asp Glu Met Ser Glu Ser Gly Gln Ser Ser Ala Ala Ala Thr
230 235 240

10 ccc agt acc aca ggt acc aag tcc aac aca ccc acg tcc tcc gtg ccc 883
Pro Ser Thr Thr Gly Thr Lys Ser Asn Thr Pro Thr Ser Ser Val Pro
15 245 250 255

20 tca gca gca gtc acg cca ctc aac gag agc cta cag ccc ctg ggg gac 931
Ser Ala Ala Val Thr Pro Leu Asn Glu Ser Leu Gln Pro Leu Gly Asp
260 265 270 275

25 tat gtc agt gtc aca aag aac aac aag cag gcc cga gag aag cgc aat 979
30 Tyr Val Ser Val Thr Lys Asn Asn Lys Gln Ala Arg Glu Lys Arg Asn
280 285 290

35 agc cgt aac atg gag gtc cag gtc acc caa gag atg cgg aac gtc agt 1027
Ser Arg Asn Met Glu Val Gln Val Thr Gln Glu Met Arg Asn Val Ser
40 295 300 305

45 atc ggc atg ggc agc agt gac gag tgg tcc gat gtt cag gac att atc 1075
Ile Gly Met Gly Ser Ser Asp Glu Trp Ser Asp Val Gln Asp Ile Ile
310 315 320

50 gac tcc acc cca gag ctg gat gtg tgt cct gaa acc cgt ctg gag cgc 1123
55 Asp Ser Thr Pro Glu Leu Asp Val Cys Pro Glu Thr Arg Leu Glu Arg
325 330 335

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5 aca gga agc agc cca acc cag gga att gta aac aaa gct ttt gga atc 1171
Thr Gly Ser Ser Pro Thr Gln Gly Ile Val Asn Lys Ala Phe Gly Ile

10 340 345 350 355

15 aac act gac tcc ttg tat cac gaa ctc tcc acg gcg gga tct gag gtc 1219
Asn Thr Asp Ser Leu Tyr His Glu Leu Ser Thr Ala Gly Ser Glu Val

20 360 365 370

25 atc ggg gat gtg gac gag gga gct gat ctc cta ggg gag ttt tca gtg 1267
Ile Gly Asp Val Asp Glu Gly Ala Asp Leu Leu Gly Glu Phe Ser Val

30 375 380 385

35 cgc gat gat ttt ttt gga atg ggc aaa gaa gtg ggg aac ctg ctg ctg 1315
Arg Asp Asp Phe Phe Gly Met Gly Lys Glu Val Gly Asn Leu Leu Leu

40 390 395 400

45 gag aac tca cag ctt cta gag aca aaa aat gct tta aat gta gtg aag 1363
Glu Asn Ser Gln Leu Leu Glu Thr Lys Asn Ala Leu Asn Val Val Lys

50 405 410 415

55 aat gac ctc att gct aag gtt gac caa ctg tca gga gaa cag gag gtc 1411
Asn Asp Leu Ile Ala Lys Val Asp Gln Leu Ser Gly Glu Gln Glu Val

420 425 430 435

ctg aag ggt gag ctg gaa gca gcc aag caa gcg aaa gtc aag ctg gag 1459

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5 Leu Lys Gly Glu Leu Glu Ala Ala Lys Gln Ala Lys Val Lys Leu Glu
 440 445 450
 10 aac cga atc aaa gag ctt gaa gaa gaa ctg aag aga gtc aag tca gag 1507
 Asn Arg Ile Lys Glu Leu Glu Glu Glu Leu Lys Arg Val Lys Ser Glu
 455 460 465
 15 gca gta act gcc cgc cgt gag ccc aga gaa gag gtg gag gat gta agc 1555
 20 Ala Val Thr Ala Arg Arg Glu Pro Arg Glu Glu Val Glu Asp Val Ser
 470 475 480
 25 agc tat ctc tgt aca gaa ttg gac aaa atc ccc atg gcc cag cgc cga 1603
 Ser Tyr Leu Cys Thr Glu Leu Asp Lys Ile Pro Met Ala Gln Arg Arg
 30 485 490 495
 35 cgc ttc aca cgg gtg gag atg gcc cga gtg ctc atg gaa cgc aac cag 1651
 Arg Phe Thr Arg Val Glu Met Ala Arg Val Leu Met Glu Arg Asn Gln
 500 505 510 515
 40 tac aag gaa cgc ctc atg gag ctg cag gag gct gtg agg tgg act gaa 1699
 45 Tyr Lys Glu Arg Leu Met Glu Leu Gln Glu Ala Val Arg Trp Thr Glu
 520 525 530
 50 atg atc aga gca tca agg gaa cac cca tct gtc cag gag aag aag aag 1747
 Met Ile Arg Ala Ser Arg Glu His Pro Ser Val Gln Glu Lys Lys Lys
 55 535 540 545

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5
 tcc acc atc tgg cag ttc ttt agt cgc ctc ttc agc tcc tca tct agc 1796
 Ser Thr Ile Trp Gln Phe Phe Ser Arg Leu Phe Ser Ser Ser Ser Ser

10
 550 555 560

15
 ccc cct ccg gcc aaa cga tcc tac cca tct gtg aac att cac tac aag 1843
 Pro Pro Pro Ala Lys Arg Ser Tyr Pro Ser Val Asn Ile His Tyr Lys

20
 565 570 575

25
 tca ccc act gca gct ggc ttt agc cag cgt cgc agc cat gct ttg tgc 1891
 Ser Pro Thr Ala Ala Gly Phe Ser Gln Arg Arg Ser His Ala Leu Cys

30
 580 585 590 595

35
 cag atc tca gcc ggc agc agg ccc ctg gag ttc ttc cct gat gat gac 1939
 Gln Ile Ser Ala Gly Ser Arg Pro Leu Glu Phe Phe Pro Asp Asp Asp

40
 600 605 610

45
 tgc acc tct tct gcc cgg cgg gag cag aag cgg gag cag tac cgc cag 1987
 Cys Thr Ser Ser Ala Arg Arg Glu Gln Lys Arg Glu Gln Tyr Arg Gln

50
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 Val Arg Glu His Val Arg Asn Asp Asp Gly Arg Leu Gln Ala Cys Gly

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5 Trp Ser Leu Pro Ala Lys Tyr Lys Gln Leu Ser Pro Asn Gly Gly Gln
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Asn Leu Ser Gly Trp Lys Pro His Glu Glu Asp Ser Ser Asn Gly Pro
30 695 700 705

35 aag cct gta cca ggt cga gac cct ctg acc tgt gac cgg gaa gga gaa 2275
Lys Pro Val Pro Gly Arg Asp Pro Leu Thr Cys Asp Arg Glu Gly Glu
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725 730 735

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Glu Thr Pro Glu Ala Asp Ala Thr Ser Ser Arg Val Trp Ile Leu Thr
55 740 745 750 755

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5 agc acc ctg aca acc agc aag gtg gtg atc att gat gcc aac cag cca 2419
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 25 Ile Ser Ser Ile Pro Ala Ala Ser Asp Ser Asp Tyr Pro Pro Gly Glu
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40 gtg ctg gct ggc atc acc ctg gtg ggg tgt gct acc cgc tgc aat gtt 2611
 Val Leu Ala Gly Ile Thr Leu Val Gly Cys Ala Thr Arg Cys Asn Val
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45 cca cgt agc aac tgt tcc tca cga gga gac acc cca gta ctg gac aag 2659
 Pro Arg Ser Asn Cys Ser Ser Arg Gly Asp Thr Pro Val Leu Asp Lys
 50 840 845 850

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	gtg gag ccc agt ggg gaa ctc tca aca aca acc agt agc gct gca ccc	2947
45	Val Glu Pro Ser Gly Glu Leu Ser Thr Thr Thr Ser Ser Ala Ala Pro	
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20 gat ggg acc ctg gct atc ttc cat cgt gga gag gat ggc cag tgg gac 3139
Asp Gly Thr Leu Ala Ile Phe His Arg Gly Glu Asp Gly Gln Trp Asp

25 1000 1005 1010

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Leu Ser Asn Tyr His Leu Met Asp Leu Gly His Pro His His Ser Ile

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5 Asp Ala His Pro Arg Arg Glu Ser Gln Val Arg Gln Leu Ala Trp Ile
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20 tac cat gct cac acc cac cag cac ctg cag gat gtg gac att gag ccc 3427
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1280

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1300

1305

1310

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 Met Met Glu
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 Ile Gln Met Asp Glu Gly Gly Gly Val Val Val Tyr Gln Asp Asp Tyr
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	ctg cgc aaa cag gcc gag gag aaa ttc atc gaa ttt gaa gat gcc ttg	451		
	Leu Arg Lys Gln Ala Glu Glu Lys Phe Ile Glu Phe Glu Asp Ala Leu			
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25				
	gaa caa gag aag aaa gaa ctc cag atc cag gta gaa cat tat gag ttt	499		
	Glu Gln Glu Lys Lys Glu Leu Gln Ile Gln Val Glu His Tyr Glu Phe			
	120	125	130	
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	cag aca cgc cag ctg gag cta aag gcc aaa aac tat gca gat cag att	547		
35	Gln Thr Arg Gln Leu Glu Leu Lys Ala Lys Asn Tyr Ala Asp Gln Ile			
	135	140	145	
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	tcc cga ctg gag gaa cga gaa tcg gag atg aag aag gaa tac aat gcc	595		
	Ser Arg Leu Glu Glu Arg Glu Ser Glu Met Lys Lys Glu Tyr Asn Ala			
45	150	155	160	
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	ctg cac cag cgg cac aca gag atg atc cag acc tat gtg gaa cac att	643		
	Leu His Gln Arg His Thr Glu Met Ile Gln Thr Tyr Val Glu His Ile			
	165	170	175	
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5 gaa aga tcc aag atg cag caa gtt ggg ggt agc ggc caa aca gaa agc 691
 Glu Arg Ser Lys Met Gln Gln Val Gly Gly Ser Gly Gln Thr Glu Ser
 180 185 190 195

10 agc ctg ccc ggg cgg agc agg aag gag cgt ccc acc tct ctg aat gtc 739
 Ser Leu Pro Gly Arg Ser Arg Lys Glu Arg Pro Thr Ser Leu Asn Val
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20 ttc ccc ctg gct gat ggc atg gta cgt gca cag atg ggg ggc aag ctc 787
 Phe Pro Leu Ala Asp Gly Met Val Arg Ala Gln Met Gly Gly Lys Leu
 25 215 220 225

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 Val Pro Ala Gly Asp His Trp His Leu Ser Asp Leu Gly Gln Leu Gln
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35 tcc agc tcc agc tac cag tgt cca aac gat gag atg tct gag tca ggc 883
 Ser Ser Ser Ser Tyr Gln Cys Pro Asn Asp Glu Met Ser Glu Ser Gly
 40 245 250 255

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 Gln Ser Ser Ala Ala Ala Thr Pro Ser Thr Thr Gly Thr Lys Ser Asn
 50 260 265 270 275

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 Thr Pro Thr Ser Ser Val Pro Ser Ala Ala Val Thr Pro Leu Asn Glu

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	295	300	305	
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	310	315	320	
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30	caa gag atg cgg aac gtc agt atc ggc atg ggc agc agt gac gag tgg Gln Glu Met Arg Asn Val Ser Ile Gly Met Gly Ser Ser Asp Glu Trp			1123
	325	330	335	
35				
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	340	345	350	355
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	360	365	370	
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	375	380	385	

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	Ser Thr Ala Gly Ser Glu Val Ile Gly Asp Val Asp Glu Gly Ala Asp	
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	Leu Leu Gly Glu Phe Ser Val Arg Asp Asp Phe Phe Gly Met Gly Lys	
20	405 410 415	
25	gaa gtg ggg aac ctg ctg ctg gag aac tca cag ctt cta gag aca aaa	1411
	Glu Val Gly Asn Leu Leu Leu Glu Asn Ser Gln Leu Leu Glu Thr Lys	
30	420 425 430 435	
35	aat gct tta aat gta gtg aag aat gac ctc att gct aag gtt gac caa	1459
	Asn Ala Leu Asn Val Val Lys Asn Asp Leu Ile Ala Lys Val Asp Gln	
40	440 445 450	
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	Leu Lys Arg Val Lys Ser Glu Ala Val Thr Ala Arg Arg Glu Pro Arg	

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5	485	490	495	
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	Glu Glu Val Glu Asp Val Ser Ser Tyr Leu Cys Thr Glu Leu Asp Lys			
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15				
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20	Ile Pro Met Ala Gln Arg Arg Arg Phe Thr Arg Val Glu Met Ala Arg			
		520	525	530
25				
	gtg ctc atg gaa cgc aac cag tac aag gaa cgc ctc atg gag ctg cag			1747
	Val Leu Met Glu Arg Asn Gln Tyr Lys Glu Arg Leu Met Glu Leu Gln			
30		535	540	545
35				
	gag gct gtg agg tgg act gaa atg atc aga gca tca agg gaa cac cca			1795
	Glu Ala Val Arg Trp Thr Glu Met Ile Arg Ala Ser Arg Glu His Pro			
	550	555	560	
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	tct gtc cag gag aag aag aag tcc acc atc tgg cag ttc ttt agt cgc			1843
	Ser Val Gln Glu Lys Lys Lys Ser Thr Ile Trp Gln Phe Phe Ser Arg			
45		565	570	575
50				
	ctc ttc agc tcc tca tct agc ccc cct ccg gcc aaa cga tcc tac cca			1891
	Leu Phe Ser Ser Ser Ser Ser Pro Pro Pro Ala Lys Arg Ser Tyr Pro			
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 Ser Val Asn Ile His Tyr Lys Ser Pro Thr Ala Ala Gly Phe Ser Gln
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15 cgt cgc agc cat gct ttg tgc cag atc tca gcc ggc agc agg ccc ctg 1987
 Arg Arg Ser His Ala Leu Cys Gln Ile Ser Ala Gly Ser Arg Pro Leu
 615 620 625

20 gag ttc ttc cct gat gat gac tgc acc tct tct gcc cgg cgg gag cag 2035
 Glu Phe Phe Pro Asp Asp Asp Cys Thr Ser Ser Ala Arg Arg Glu Gln
 25 630 635 640

30 aag cgg gag cag tac cgc cag gtt cgt gaa cac gtg cgc aat gat gac 2083
 Lys Arg Glu Gln Tyr Arg Gln Val Arg Glu His Val Arg Asn Asp Asp
 35 645 650 655

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	Glu Asp Ser Ser Asn Gly Pro Lys Pro Val Pro Gly Arg Asp Pro Leu			
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	agt cgg gta tgg atc ctc acc agc acc ctg aca acc agc aag gtg gtg	2467		
	Ser Arg Val Trp Ile Leu Thr Ser Thr Leu Thr Thr Ser Lys Val Val			
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	atc att gat gcc aac cag cca ggc aca att gtg gat cag ttc aca gtc	2515		
	Ile Ile Asp Ala Asn Gln Pro Gly Thr Ile Val Asp Gln Phe Thr Val			
	790	795	800	
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	805 810 815	
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	Glu Asp Ser Gly Ala Asp Gly Val Leu Ala Gly Ile Thr Leu Val Gly	
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	Cys Ala Thr Arg Cys Asn Val Pro Arg Ser Asn Cys Ser Ser Arg Gly	
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	870 875 880	
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	Asn Gly Lys Val Asn Pro Ser Gln Ser Thr Glu Glu Ala Thr Glu Ala	
50	885 890 895	
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15	cca tcc tcc agc acc cag cct gcc agt gag aat ggg tca gaa tcc aat				2947
	Pro Ser Ser Ser Thr Gln Pro Ala Ser Glu Asn Gly Ser Glu Ser Asn				
20	935	940	945		
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	Gly Thr Ile Val Gln Pro Gln Val Glu Pro Ser Gly Glu Leu Ser Thr				
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15	aag ctg ggc ttc tcc ttc gtg cgc atc aca gcc tta ctc att gca ggc	3571		
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25	aac cgt ctg tgg gtg ggc act ggc aat ggg gtt gtc atc tcc atc ccc	3619		
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	Ile Ile His Val Tyr Gly Asp Asp Ser Ser Asp Lys Ala Ala Ser Ser			
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Met Met Glu

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 30 245 250 255

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50 cta cag ccc ctg ggg gac tat gtc agt gtc aca aag aac aac aag cag 1027
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 55 295 300 305

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30 340 345 350 355

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40 360 365 370

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50 375 380 385

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EP 1 134 230 A1

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15 420 425 430 435

20 gct tta aat gta gtg aag aat gac ctc att gct aag gtt gac caa ctg 1459
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Ala Lys Val Lys Leu Glu Asn Arg Ile Lys Glu Leu Glu Glu Glu Leu
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EP 1 134 230 A1

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EP 1 134 230 A1

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25 645 650 655

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55 695 700 705

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710 715 720

EP 1 134 230 A1

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EP 1 134 230 A1

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 20 Ala Thr Arg Cys Asn Val Pro Arg Ser Asn Cys Ser Ser Arg Gly Asp
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 Pro Pro Ile Met Asn Gly Pro Met His Pro Arg Pro Leu Val Ala Leu

10 15 20

50 ctg gat ggc cgg gac tgc aca gtg gag atg cct atc ctg aag gat gtg 151
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	Lys Val Leu Asn Glu Ala Val Gly Ala Leu Met Tyr His Thr Ile Thr			
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40	cta ggc atc gca gtg tgc aat gtg ccg gca gca tct gtg gaa gaa acg 391			
	Leu Gly Ile Ala Val Cys Asn Val Pro Ala Ala Ser Val Glu Glu Thr			
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 Asp Gly Ile Glu Arg Ala Leu Gly Leu Gln Arg Val Ser Thr Leu Gln
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 215 220 225

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 Glu His Asn His His Leu Ile Asn Asp Phe Thr Val Lys Gln Met Arg
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	Lys Ala Val Ala Gln Ala Leu Lys Glu Gly Arg Ile Arg Gly Ala Ala				
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 Tyr Leu Val Thr Gly Glu Ser Gly His Met Pro Ala Val Arg Val Trp
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EP 1 134 230 A1

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EP 1 134 230 A1

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 Pro Lys Lys Val Gly Lys Val Tyr Ser Ala Leu Tyr His Ser Ser Cys

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EP 1 134 230 A1

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EP 1 134 230 A1

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EP 1 134 230 A1

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EP 1 134 230 A1

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EP 1 134 230 A1

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EP 1 134 230 A1

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EP 1 134 230 A1

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EP 1 134 230 A1

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EP 1 134 230 A1

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	210	215	220	
50	aag cct tcc cag gtg cct gca gtg gag gtg att cct gca gga gcc tcc	720		
	Lys Pro Ser Gln Val Pro Ala Val Glu Val Ile Pro Ala Gly Ala Ser			
55	225	230	235	240

55

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5	Leu Glu Asp Val Arg Leu Gln Glu Arg Thr Thr Gly Gly Leu Leu Ala	
	65 70 75	
10	gag gcc cca aac gaa aag ctc ttc ttc gtg gac aca gga ttc aag aga	291
	Glu Ala Pro Asn Glu Lys Leu Phe Phe Val Asp Thr Gly Phe Lys Arg	
	80 85 90 95	
15	aaa gaa cca aga aag aag agg acc ttg gtc cag aag aag tca cag cgt	339
	Lys Glu Pro Arg Lys Lys Arg Thr Leu Val Gln Lys Lys Ser Gln Arg	
	100 105 110	
20	ctc cag aaa ccc tta cgg gtt gac ctt gcc ctt gag aat cat tct aag	387
	Leu Gln Lys Pro Leu Arg Val Asp Leu Ala Leu Glu Asn His Ser Lys	
	115 120 125	
25	atc cct gct ccc aaa gac atc ctc gca cat cag gtc cct aat gcc aag	435
	Ile Pro Ala Pro Lys Asp Ile Leu Ala His Gln Val Pro Asn Ala Lys	
	130 135 140	
30	aag ctc agg cga aag gag gag tta tgg gag aaa ctg gca aag cag ggc	483
	Lys Leu Arg Arg Lys Glu Glu Leu Trp Glu Lys Leu Ala Lys Gln Gly	
	145 150 155	
35	gaa ctg ccc agg gat gtg cgc aag gca cag gcc cga ctc ctt agc cct	531
	Glu Leu Pro Arg Asp Val Arg Lys Ala Gln Ala Arg Leu Leu Ser Pro	
	160 165 170 175	
40	ccc aca cca aag gcc aaa cct ggg ccc cag gac atc att gag cga ccc	579
	Pro Thr Pro Lys Ala Lys Pro Gly Pro Gln Asp Ile Ile Glu Arg Pro	
45	180 185 190	
50	ttc tat gac ctc tgg aac cca gac aac cct ctg gac acg cct ttg att	627
	Phe Tyr Asp Leu Trp Asn Pro Asp Asn Pro Leu Asp Thr Pro Leu Ile	
	195 200 205	
55	ggc cag gat gca ttt ttt ctg gaa cag acc aag aag aaa ggc gtg agg	675
	Gly Gln Asp Ala Phe Phe Leu Glu Gln Thr Lys Lys Lys Gly Val Arg	

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5	210	215	220	
	cgg cca caa cgc ctc cac atc aag cct tcc cag gtg cct gca gtg gag	723		
10	Arg Pro Gln Arg Leu His Ile Lys Pro Ser Gln Val Pro Ala Val Glu			
	225	230	235	
	gtg att cct gca gga gcc tcc tac aac cca acc ttt gaa gat cac cag	771		
15	Val Ile Pro Ala Gly Ala Ser Tyr Asn Pro Thr Phe Glu Asp His Gln			
	240	245	250	255
	gcc ctg ctt cga gag gcc cat gag gtg gag ctg cag cgt gag aaa gag	819		
20	Ala Leu Leu Arg Glu Ala His Glu Val Glu Leu Gln Arg Glu Lys Glu			
	260	265	270	
	gca gaa aag ctg gag cga cag ctg gcc ctg ccc acc tca gag caa gct	867		
25	Ala Glu Lys Leu Glu Arg Gln Leu Ala Leu Pro Thr Ser Glu Gln Ala			
	275	280	285	
	gcc acc cag gag tcc gtg ttt cgg gag atg tgt gag ggc ctg ctg gag	915		
30	Ala Thr Gln Glu Ser Val Phe Arg Glu Met Cys Glu Gly Leu Leu Glu			
	290	295	300	
	gag tct gat ggt gag gat gag cat gag gca ggc cgt gcc ggg cag cca	963		
35	Glu Ser Asp Gly Glu Asp Glu His Glu Ala Gly Arg Ala Gly Gln Pro			
	305	310	315	
	gag gct ggt gat ggg acc acc gag atc tca ccc act ggt gct gct ggt	1011		
40	Glu Ala Gly Asp Gly Thr Thr Glu Ile Ser Pro Thr Gly Ala Ala Gly			
	320	325	330	335
	cct gag aag agg atg gag aag aag acg gag cag cag cgg cgg cgg gag	1059		
50	Pro Glu Lys Arg Met Glu Lys Lys Thr Glu Gln Gln Arg Arg Arg Glu			
	340	345	350	
	aaa gct gct cgc aag ctg cgg gtg cag cag gct gca ctg agg gca gcc	1107		
55	Lys Ala Ala Arg Lys Leu Arg Val Gln Gln Ala Ala Leu Arg Ala Ala			
	355	360	365	

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5 cgg ctt cag cac caa gaa ctt ttc agg ctg cgt ggg atc aag gcc cag 1155
 Arg Leu Gln His Gln Glu Leu Phe Arg Leu Arg Gly Ile Lys Ala Gln
 370 375 380

10 gtg gcc cga agg ctg gca gaa ctg gca cgc cgg agg gag cag cgg cgc 1203
 Val Ala Arg Arg Leu Ala Glu Leu Ala Arg Arg Arg Glu Gln Arg Arg
 385 390 395

15 ata cgg cga ctg gca gag gct gac aag ccc cga agg ctg gga cgg ctc 1251
 Ile Arg Arg Leu Ala Glu Ala Asp Lys Pro Arg Arg Leu Gly Arg Leu
 400 405 410 415

20 aag tac cag gct cct gac att gat gtg cag ctc agc tct gag ttg tct 1299
 Lys Tyr Gln Ala Pro Asp Ile Asp Val Gln Leu Ser Ser Glu Leu Ser
 25 420 425 430

30 ggc tca ctc agg aca ctg aag cca gaa ggt cac att ctc cga gac agg 1347
 Gly Ser Leu Arg Thr Leu Lys Pro Glu Gly His Ile Leu Arg Asp Arg
 435 440 445

35 ttc aag agc ttc cag aag aga aat atg att gag ccc cga gaa cga gcc 1395
 Phe Lys Ser Phe Gln Lys Arg Asn Met Ile Glu Pro Arg Glu Arg Ala
 450 455 460

40 aag ttc aag cgc aaa tac aaa gtg aag ctg gtg gag aag cgg gcc tac 1443
 Lys Phe Lys Arg Lys Tyr Lys Val Lys Leu Val Glu Lys Arg Ala Tyr
 465 470 475

45 cgt gag att cag ttg tag ctgtgcagat g 1469
 Arg Glu Ile Gln Leu
 480

50

<210> 9

55 <211> 1305

5 <212> PRT
 <213> Mouse

 10 <400> 9
 Met Met Glu Ile Gln Met Asp Glu Gly Gly Gly Val Val Val Tyr Gln
 15 1 5 10 15

 20 Asp Asp Tyr Cys Ser Gly Ser Val Met Ser Glu Arg Val Ser Gly Leu
 20 25 30

 25 Ala Gly Ser Ile Tyr Arg Glu Phe Glu Arg Leu Ile His Cys Tyr Asp
 35 40 45

 30 Glu Glu Val Val Lys Glu Leu Met Pro Leu Val Val Asn Val Leu Glu
 50 55 60

 35 Asn Leu Asp Ser Val Leu Ser Glu Asn Gln Glu His Glu Val Glu Leu
 65 70 75 80

 40 Glu Leu Leu Arg Glu Asp Asn Glu Gln Leu Leu Thr Gln Tyr Glu Arg
 45 85 90 95

 50 Glu Lys Ala Leu Arg Lys Gln Ala Glu Glu Lys Phe Ile Glu Phe Glu
 100 105 110

 55 Asp Ala Leu Glu Gln Glu Lys Lys Glu Leu Gln Ile Gln Val Glu His

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5	115	120	125
10	Tyr Glu Phe Gln Thr Arg Gln Leu Glu Leu Lys Ala Lys Asn Tyr Ala		
	130	135	140
15	Asp Gln Ile Ser Arg Leu Glu Glu Arg Glu Ser Glu Met Lys Lys Glu		
	145	150	155 160
20	Tyr Asn Ala Leu His Gln Arg His Thr Glu Met Ile Gln Thr Tyr Val		
	165	170	175
25	Glu His Ile Glu Arg Ser Lys Met Gln Gln Val Gly Gly Ser Gly Gln		
30	180	185	190
35	Thr Glu Ser Ser Leu Pro Gly Arg Arg Lys Glu Arg Pro Thr Ser Leu		
	195	200	205
40	Asn Val Phe Pro Leu Ala Asp Gly Met Cys Pro Asn Asp Glu Met Ser		
	210	215	220
45	Glu Ser Gly Gln Ser Ser Ala Ala Ala Thr Pro Ser Thr Thr Gly Thr		
	225	230	235 240
50	Lys Ser Asn Thr Pro Thr Ser Ser Val Pro Ser Ala Ala Val Thr Pro		
55	245	250	255

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5	Leu Asn Glu Ser Leu Gln Pro Leu Gly Asp Tyr Val Ser Val Thr Lys	260	265	270
10	Asn Asn Lys Gln Ala Arg Glu Lys Arg Asn Ser Arg Asn Met Glu Val	275	280	285
15	Gln Val Thr Gln Glu Met Arg Asn Val Ser Ile Gly Met Gly Ser Ser	290	295	300
20	Asp Glu Trp Ser Asp Val Gln Asp Ile Ile Asp Ser Thr Pro Glu Leu	305	310	315
25				320
30	Asp Val Cys Pro Glu Thr Arg Leu Glu Arg Thr Gly Ser Ser Pro Thr	325	330	335
35	Gln Gly Ile Val Asn Lys Ala Phe Gly Ile Asn Thr Asp Ser Leu Tyr	340	345	350
40	His Glu Leu Ser Thr Ala Gly Ser Glu Val Ile Gly Asp Val Asp Glu	355	360	365
45	Gly Ala Asp Leu Leu Gly Glu Phe Ser Val Arg Asp Asp Phe Phe Gly	370	375	380
50				
55	Met Gly Lys Glu Val Gly Asn Leu Leu Leu Glu Asn Ser Gln Leu Leu	385	390	395
				400

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5
 Glu Thr Lys Asn Ala Leu Asn Val Val Lys Asn Asp Leu Ile Ala Lys
 405 410 415

10
 Val Asp Gln Leu Ser Gly Glu Gln Glu Val Leu Lys Gly Glu Leu Glu
 15 420 425 430

20
 Ala Ala Lys Gln Ala Lys Val Lys Leu Glu Asn Arg Ile Lys Glu Leu
 435 440 445

25
 Glu Glu Glu Leu Lys Arg Val Lys Ser Glu Ala Val Thr Ala Arg Arg
 450 455 460

30
 Glu Pro Arg Glu Glu Val Glu Asp Val Ser Ser Tyr Leu Cys Thr Glu
 465 470 475 480

35
 Leu Asp Lys Ile Pro Met Ala Gln Arg Arg Arg Phe Thr Arg Val Glu
 485 490 495

40
 Met Ala Arg Val Leu Met Glu Arg Asn Gln Tyr Lys Glu Arg Leu Met
 45 500 505 510

50
 Glu Leu Gln Glu Ala Val Arg Trp Thr Glu Met Ile Arg Ala Ser Arg
 515 520 525

55
 Glu His Pro Ser Val Gln Glu Lys Lys Lys Ser Thr Ile Trp Gln Phe

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	530	535	540	
5				
	Phe Ser Arg Leu Phe Ser Ser Ser Ser Ser Pro Pro Pro Ala Lys Arg			
10	545	550	555	560
	Ser Tyr Pro Ser Val Asn Ile His Tyr Lys Ser Pro Thr Ala Ala Gly			
15		565	570	575
	Phe Ser Gln Arg Arg Ser His Ala Leu Cys Gln Ile Ser Ala Gly Ser			
20		580	585	590
	Arg Pro Leu Glu Phe Phe Pro Asp Asp Asp Cys Thr Ser Ser Ala Arg			
25		595	600	605
	Arg Glu Gln Lys Arg Glu Gln Tyr Arg Gln Val Arg Glu His Val Arg			
30		610	615	620
	Asn Asp Asp Gly Arg Leu Gln Ala Cys Gly Trp Ser Leu Pro Ala Lys			
35		625	630	635
	Tyr Lys Gln Leu Ser Pro Asn Gly Gly Gln Glu Asp Thr Arg Met Lys			
40		645	650	655
	Asn Val Pro Val Pro Val Tyr Cys Arg Pro Leu Val Glu Lys Asp Pro			
45		660	665	670
50				
55				

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5	Ser Thr Lys Leu Trp Cys Ala Ala Gly Val Asn Leu Ser Gly Trp Lys	675	680	685
10	Pro His Glu Glu Asp Ser Ser Asn Gly Pro Lys Pro Val Pro Gly Arg	690	695	700
15	Asp Pro Leu Thr Cys Asp Arg Glu Gly Glu Gly Glu Pro Lys Ser Thr	705	710	715
20				720
25	His Pro Ser Pro Glu Lys Lys Lys Ala Lys Glu Thr Pro Glu Ala Asp	725	730	735
30	Ala Thr Ser Ser Arg Val Trp Ile Leu Thr Ser Thr Leu Thr Thr Ser	740	745	750
35	Lys Val Val Ile Ile Asp Ala Asn Gln Pro Gly Thr Ile Val Asp Gln	755	760	765
40	Phe Thr Val Cys Asn Ala His Val Leu Cys Ile Ser Ser Ile Pro Ala	770	775	780
45	Ala Ser Asp Ser Asp Tyr Pro Pro Gly Glu Met Phe Leu Asp Ser Asp	785	790	795
50				800
55	Val Asn Pro Glu Asp Ser Gly Ala Asp Gly Val Leu Ala Gly Ile Thr	805	810	815

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5 Leu Val Gly Cys Ala Thr Arg Cys Asn Val Pro Arg Ser Asn Cys Ser
820 825 830

10 Ser Arg Gly Asp Thr Pro Val Leu Asp Lys Gly Gln Gly Asp Val Ala
835 840 845

15 Thr Thr Ala Asn Gly Lys Val Asn Pro Ser Gln Ser Thr Glu Glu Ala
850 855 860

20 Thr Glu Ala Thr Glu Val Pro Asp Pro Gly Pro Ser Glu Ser Glu Ala
865 870 875 880

25 Thr Thr Val Arg Pro Gly Pro Leu Thr Glu His Val Phe Thr Asp Pro
885 890 895

30 Ala Pro Thr Pro Ser Ser Ser Thr Gln Pro Ala Ser Glu Asn Gly Ser
900 905 910

35 Glu Ser Asn Gly Thr Ile Val Gln Pro Gln Val Glu Pro Ser Gly Glu
915 920 925

40 Leu Ser Thr Thr Thr Ser Ser Ala Ala Pro Thr Met Trp Leu Gly Ala
930 935 940

45 Gln Asn Gly Trp Leu Tyr Val His Ser Ala Val Ala Asn Trp Lys Lys

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5	945	950	955	960
10	Cys Leu His Ser Ile Lys Leu Lys Asp Ser Val Leu Ser Leu Val His			
	965	970	975	
15	Val Lys Gly Arg Val Leu Val Ala Leu Ala Asp Gly Thr Leu Ala Ile			
	980	985	990	
20	Phe His Arg Gly Glu Asp Gly Gln Trp Asp Leu Ser Asn Tyr His Leu			
	995	1000	1005	
25	Met Asp Leu Gly His Pro His His Ser Ile Arg Cys Met Ala Val Val			
30	1010	1015	1020	
35	Asn Asp Arg Val Trp Cys Gly Tyr Lys Asn Lys Val His Val Ile Gln			
	025	1030	1035	1040
40	Pro Lys Thr Met Gln Ile Glu Lys Ser Phe Asp Ala His Pro Arg Arg			
	1045	1050	1055	
45	Glu Ser Gln Val Arg Gln Leu Ala Trp Ile Gly Asp Gly Val Trp Val			
	1060	1065	1070	
50	Ser Ile Arg Leu Asp Ser Thr Leu Arg Leu Tyr His Ala His Thr His			
55	1075	1080	1085	

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5 Gln His Leu Gln Asp Val Asp Ile Glu Pro Tyr Val Ser Lys Met Leu
1090 1095 1100

10 Gly Thr Gly Lys Leu Gly Phe Ser Phe Val Arg Ile Thr Ala Leu Leu
105 1110 1115 1120

15 Ile Ala Gly Asn Arg Leu Trp Val Gly Thr Gly Asn Gly Val Val Ile
1125 1130 1135

20 Ser Ile Pro Leu Thr Glu Thr Val Val Leu His Arg Gly Gln Leu Leu
1140 1145 1150

25 Gly Leu Arg Ala Asn Lys Thr Ser Pro Thr Ser Gly Glu Gly Thr Arg
1155 1160 1165

30 Pro Gly Gly Ile Ile His Val Tyr Gly Asp Asp Ser Ser Asp Lys Ala
1170 1175 1180

35 Ala Ser Ser Phe Ile Pro Tyr Cys Ser Met Ala Gln Ala Gln Leu Cys
185 1190 1195 1200

40 Phe His Gly His Arg Asp Ala Val Lys Phe Phe Val Ser Val Pro Gly
1205 1210 1215

45 Asn Val Leu Ala Thr Leu Asn Gly Ser Val Leu Asp Ser Pro Ser Glu
1220 1225 1230

50

55

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5

Gly Pro Gly Pro Ala Ala Pro Ala Ala Asp Ala Glu Gly Gln Lys Leu
1235 1240 1245

10

Lys Asn Ala Leu Val Leu Ser Gly Gly Glu Gly Tyr Ile Asp Phe Arg
1250 1255 1260

15

Ile Gly Asp Gly Glu Asp Asp Glu Thr Glu Glu Cys Ala Gly Asp Val
265 1270 1275 1280

20

Asn Gln Thr Lys Pro Ser Leu Ser Lys Ala Glu Arg Ser His Ile Ile
1285 1290 1295

25

Val Trp Gln Val Ser Tyr Thr Pro Glu
1300 1305

30

35

<210> 10

40

<211> 1314

<212> PRT

45

<213> Mouse

<400> 10

50

Met Met Glu Ile Gln Met Asp Glu Gly Gly Gly Val Val Val Tyr Gln
1 5 10 15

55

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5	Asp Asp Tyr Cys Ser Gly Ser Val Met Ser Glu Arg Val Ser Gly Leu			
	20	25	30	
10	Ala Gly Ser Ile Tyr Arg Glu Phe Glu Arg Leu Ile His Cys Tyr Asp			
	35	40	45	
15	Glu Glu Val Val Lys Glu Leu Met Pro Leu Val Val Asn Val Leu Glu			
	50	55	60	
20	Asn Leu Asp Ser Val Leu Ser Glu Asn Gln Glu His Glu Val Glu Leu			
25	65	70	75	80
	Glu Leu Leu Arg Glu Asp Asn Glu Gln Leu Leu Thr Gln Tyr Glu Arg			
30	85	90	95	
	Glu Lys Ala Leu Arg Lys Gln Ala Glu Glu Lys Phe Ile Glu Phe Glu			
35	100	105	110	
40	Asp Ala Leu Glu Gln Glu Lys Lys Glu Leu Gln Ile Gln Val Glu His			
	115	120	125	
45	Tyr Glu Phe Gln Thr Arg Gln Leu Glu Leu Lys Ala Lys Asn Tyr Ala			
	130	135	140	
50	Asp Gln Ile Ser Arg Leu Glu Glu Arg Glu Ser Glu Met Lys Lys Glu			
55	145	150	155	160

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5 Tyr Asn Ala Leu His Gln Arg His Thr Glu Met Ile Gln Thr Tyr Val
165 170 175

10 Glu His Ile Glu Arg Ser Lys Met Gln Gln Val Gly Gly Ser Gly Gln
180 185 190

15 Thr Glu Ser Ser Leu Pro Gly Arg Ser Pro Arg Gln Ser Trp Arg Lys
195 200 205

20 Ser Arg Lys Glu Arg Pro Thr Ser Leu Asn Val Phe Pro Leu Ala Asp
210 215 220

25 Gly Met Cys Pro Asn Asp Glu Met Ser Glu Ser Gly Gln Ser Ser Ala
225 230 235 240

30 Ala Ala Thr Pro Ser Thr Thr Gly Thr Lys Ser Asn Thr Pro Thr Ser
245 250 255

35 Ser Val Pro Ser Ala Ala Val Thr Pro Leu Asn Glu Ser Leu Gln Pro
260 265 270

40 Leu Gly Asp Tyr Val Ser Val Thr Lys Asn Asn Lys Gln Ala Arg Glu
275 280 285

45 Lys Arg Asn Ser Arg Asn Met Glu Val Gln Val Thr Gln Glu Met Arg

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	290	295	300	
5				
	Asn Val Ser Ile Gly Met Gly Ser Ser Asp Glu Trp Ser Asp Val Gln			
10	305	310	315	320
15	Asp Ile Ile Asp Ser Thr Pro Glu Leu Asp Val Cys Pro Glu Thr Arg			
	325	330	335	
20	Leu Glu Arg Thr Gly Ser Ser Pro Thr Gln Gly Ile Val Asn Lys Ala			
	340	345	350	
25	Phe Gly Ile Asn Thr Asp Ser Leu Tyr His Glu Leu Ser Thr Ala Gly			
	355	360	365	
30	Ser Glu Val Ile Gly Asp Val Asp Glu Gly Ala Asp Leu Leu Gly Glu			
	370	375	380	
35	Phe Ser Val Arg Asp Asp Phe Phe Gly Met Gly Lys Glu Val Gly Asn			
	385	390	395	400
40	Leu Leu Leu Glu Asn Ser Gln Leu Leu Glu Thr Lys Asn Ala Leu Asn			
	405	410	415	
45	Val Val Lys Asn Asp Leu Ile Ala Lys Val Asp Gln Leu Ser Gly Glu			
	420	425	430	
50				
55				

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5	Gln Glu Val Leu Lys Gly Glu Leu Glu Ala Ala Lys Gln Ala Lys Val			
	435	440	445	
10	Lys Leu Glu Asn Arg Ile Lys Glu Leu Glu Glu Glu Leu Lys Arg Val			
	450	455	460	
15	Lys Ser Glu Ala Val Thr Ala Arg Arg Glu Pro Arg Glu Glu Val Glu			
20	465	470	475	480
25	Asp Val Ser Ser Tyr Leu Cys Thr Glu Leu Asp Lys Ile Pro Met Ala			
	485	490	495	
30	Gln Arg Arg Arg Phe Thr Arg Val Glu Met Ala Arg Val Leu Met Glu			
	500	505	510	
35	Arg Asn Gln Tyr Lys Glu Arg Leu Met Glu Leu Gln Glu Ala Val Arg			
	515	520	525	
40	Trp Thr Glu Met Ile Arg Ala Ser Arg Glu His Pro Ser Val Gln Glu			
	530	535	540	
45	Lys Lys Lys Ser Thr Ile Trp Gln Phe Phe Ser Arg Leu Phe Ser Ser			
50	545	550	555	560
55	Ser Ser Ser Pro Pro Pro Ala Lys Arg Ser Tyr Pro Ser Val Asn Ile			
	565	570	575	

5 His Tyr Lys Ser Pro Thr Ala Ala Gly Phe Ser Gln Arg Arg Ser His
580 585 590

10 Ala Leu Cys Gln Ile Ser Ala Gly Ser Arg Pro Leu Glu Phe Phe Pro
595 600 605

15 Asp Asp Asp Cys Thr Ser Ser Ala Arg Arg Glu Gln Lys Arg Glu Gln
20 610 615 620

25 Tyr Arg Gln Val Arg Glu His Val Arg Asn Asp Asp Gly Arg Leu Gln
625 630 635 640

30 Ala Cys Gly Trp Ser Leu Pro Ala Lys Tyr Lys Gln Leu Ser Pro Asn
645 650 655

35 Gly Gly Gln Glu Asp Thr Arg Met Lys Asn Val Pro Val Pro Val Tyr
660 665 670

40 Cys Arg Pro Leu Val Glu Lys Asp Pro Ser Thr Lys Leu Trp Cys Ala
675 680 685

45 Ala Gly Val Asn Leu Ser Gly Trp Lys Pro His Glu Glu Asp Ser Ser
690 695 700

50 Asn Gly Pro Lys Pro Val Pro Gly Arg Asp Pro Leu Thr Cys Asp Arg
55

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5	705	710	715	720
	Glu Gly Glu Gly Glu Pro Lys Ser Thr His Pro Ser Pro Glu Lys Lys			
10		725	730	735
	Lys Ala Lys Glu Thr Pro Glu Ala Asp Ala Thr Ser Ser Arg Val Trp			
15		740	745	750
	Ile Leu Thr Ser Thr Leu Thr Thr Ser Lys Val Val Ile Ile Asp Ala			
20		755	760	765
	Asn Gln Pro Gly Thr Ile Val Asp Gln Phe Thr Val Cys Asn Ala His			
25		770	775	780
	Val Leu Cys Ile Ser Ser Ile Pro Ala Ala Ser Asp Ser Asp Tyr Pro			
30		785	790	795
				800
	Pro Gly Glu Met Phe Leu Asp Ser Asp Val Asn Pro Glu Asp Ser Gly			
40		805	810	815
	Ala Asp Gly Val Leu Ala Gly Ile Thr Leu Val Gly Cys Ala Thr Arg			
45		820	825	830
	Cys Asn Val Pro Arg Ser Asn Cys Ser Ser Arg Gly Asp Thr Pro Val			
50		835	840	845
55				

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5 Leu Asp Lys Gly Gln Gly Asp Val Ala Thr Thr Ala Asn Gly Lys Val
 850 855 860
 10 Asn Pro Ser Gln Ser Thr Glu Glu Ala Thr Glu Ala Thr Glu Val Pro
 865 870 875 880
 15 Asp Pro Gly Pro Ser Glu Ser Glu Ala Thr Thr Val Arg Pro Gly Pro
 885 890 895
 20 Leu Thr Glu His Val Phe Thr Asp Pro Ala Pro Thr Pro Ser Ser Ser
 25 900 905 910
 30 Thr Gln Pro Ala Ser Glu Asn Gly Ser Glu Ser Asn Gly Thr Ile Val
 915 920 925
 35 Gln Pro Gln Val Glu Pro Ser Gly Glu Leu Ser Thr Thr Thr Ser Ser
 930 935 940
 40 Ala Ala Pro Thr Met Trp Leu Gly Ala Gln Asn Gly Trp Leu Tyr Val
 945 950 955 960
 45 His Ser Ala Val Ala Asn Trp Lys Lys Cys Leu His Ser Ile Lys Leu
 965 970 975
 50 Lys Asp Ser Val Leu Ser Leu Val His Val Lys Gly Arg Val Leu Val
 55 980 985 990

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5 Ala Leu Ala Asp Gly Thr Leu Ala Ile Phe His Arg Gly Glu Asp Gly
995 1000 1005

10 Gln Trp Asp Leu Ser Asn Tyr His Leu Met Asp Leu Gly His Pro His
1010 1015 1020

15 His Ser Ile Arg Cys Met Ala Val Val Asn Asp Arg Val Trp Cys Gly
20 025 1030 1035 1040

25 Tyr Lys Asn Lys Val His Val Ile Gln Pro Lys Thr Met Gln Ile Glu
1045 1050 1055

30 Lys Ser Phe Asp Ala His Pro Arg Arg Glu Ser Gln Val Arg Gln Leu
1060 1065 1070

35 Ala Trp Ile Gly Asp Gly Val Trp Val Ser Ile Arg Leu Asp Ser Thr
1075 1080 1085

40 Leu Arg Leu Tyr His Ala His Thr His Gln His Leu Gln Asp Val Asp
1090 1095 1100

45 Ile Glu Pro Tyr Val Ser Lys Met Leu Gly Thr Gly Lys Leu Gly Phe
50 105 1110 1115 1120

55 Ser Phe Val Arg Ile Thr Ala Leu Leu Ile Ala Gly Asn Arg Leu Trp

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5	1125	1130	1135
	Val Gly Thr Gly Asn Gly Val Val Ile Ser Ile Pro Leu Thr Glu Thr		
10	1140	1145	1150
	Val Val Leu His Arg Gly Gln Leu Leu Gly Leu Arg Ala Asn Lys Thr		
15	1155	1160	1165
	Ser Pro Thr Ser Gly Glu Gly Thr Arg Pro Gly Gly Ile Ile His Val		
20	1170	1175	1180
	Tyr Gly Asp Asp Ser Ser Asp Lys Ala Ala Ser Ser Phe Ile Pro Tyr		
25	185	1190	1195 1200
	Cys Ser Met Ala Gln Ala Gln Leu Cys Phe His Gly His Arg Asp Ala		
30	1205	1210	1215
	Val Lys Phe Phe Val Ser Val Pro Gly Asn Val Leu Ala Thr Leu Asn		
35	1220	1225	1230
	Gly Ser Val Leu Asp Ser Pro Ser Glu Gly Pro Gly Pro Ala Ala Pro		
40	1235	1240	1245
	Ala Ala Asp Ala Glu Gly Gln Lys Leu Lys Asn Ala Leu Val Leu Ser		
45	1250	1255	1260
50			
55			

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5 Gly Gly Glu Gly Tyr Ile Asp Phe Arg Ile Gly Asp Gly Glu Asp Asp
265 1270 1275 1280

10 Glu Thr Glu Glu Cys Ala Gly Asp Val Asn Gln Thr Lys Pro Ser Leu
1285 1290 1295

15 Ser Lys Ala Glu Arg Ser His Ile Ile Val Trp Gln Val Ser Tyr Thr
1300 1305 1310

20 Pro Glu

25

30 <210> 11
<211> 1337
<212> PRT

35 <213> Mouse

40 <400> 11
Met Met Glu Ile Gln Met Asp Glu Gly Gly Gly Val Val Val Tyr Gln
1 5 10 15

45 Asp Asp Tyr Cys Ser Gly Ser Val Met Ser Glu Arg Val Ser Gly Leu
50 20 25 30

55 Ala Gly Ser Ile Tyr Arg Glu Phe Glu Arg Leu Ile His Cys Tyr Asp

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5	35	40	45
	Glu. Glu Val Val Lys Glu Leu Met Pro Leu Val Val Asn Val Leu Glu		
10	50	55	60
	Asn Leu Asp Ser Val Leu Ser Glu Asn Gln Glu His Glu Val Glu Leu		
15	65	70	75 80
	Glu Leu Leu Arg Glu Asp Asn Glu Gln Leu Leu Thr Gln Tyr Glu Arg		
20	85	90	95
	Glu Lys Ala Leu Arg Lys Gln Ala Glu Glu Lys Phe Ile Glu Phe Glu		
25	100	105	110
	Asp Ala Leu Glu Gln Glu Lys Lys Glu Leu Gln Ile Gln Val Glu His		
30	115	120	125
	Tyr Glu Phe Gln Thr Arg Gln Leu Glu Leu Lys Ala Lys Asn Tyr Ala		
35	130	135	140
	Asp Gln Ile Ser Arg Leu Glu Glu Arg Glu Ser Glu Met Lys Lys Glu		
40	145	150	155 160
	Tyr Asn Ala Leu His Gln Arg His Thr Glu Met Ile Gln Thr Tyr Val		
45	165	170	175
50			
55			

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5	Glu His Ile Glu Arg Ser Lys Met Gln Gln Val Gly Gly Ser Gly Gln	180	185	190
10	Thr Glu Ser Ser Leu Pro Gly Arg Ser Arg Lys Glu Arg Pro Thr Ser	195	200	205
15	Leu Asn Val Phe Pro Leu Ala Asp Gly Met Val Arg Ala Gln Met Gly	210	215	220
20	Gly Lys Leu Val Pro Ala Gly Asp His Trp His Leu Ser Asp Leu Gly	225	230	235
25				240
30	Gln Leu Gln Ser Ser Ser Ser Tyr Gln Cys Pro Asn Asp Glu Met Ser	245	250	255
35	Glu Ser Gly Gln Ser Ser Ala Ala Ala Thr Pro Ser Thr Thr Gly Thr	260	265	270
40	Lys Ser Asn Thr Pro Thr Ser Ser Val Pro Ser Ala Ala Val Thr Pro	275	280	285
45	Leu Asn Glu Ser Leu Gln Pro Leu Gly Asp Tyr Val Ser Val Thr Lys	290	295	300
50	Asn Asn Lys Gln Ala Arg Glu Lys Arg Asn Ser Arg Asn Met Glu Val	305	310	315
55				320

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5 Gln Val Thr Gln Glu Met Arg Asn Val Ser Ile Gly Met Gly Ser Ser
325 330 335

10 Asp Glu Trp Ser Asp Val Gln Asp Ile Ile Asp Ser Thr Pro Glu Leu
340 345 350

15 Asp Val Cys Pro Glu Thr Arg Leu Glu Arg Thr Gly Ser Ser Pro Thr
20 355 360 365

25 Gln Gly Ile Val Asn Lys Ala Phe Gly Ile Asn Thr Asp Ser Leu Tyr
370 375 380

30 His Glu Leu Ser Thr Ala Gly Ser Glu Val Ile Gly Asp Val Asp Glu
385 390 395 400

35 Gly Ala Asp Leu Leu Gly Glu Phe Ser Val Arg Asp Asp Phe Phe Gly
405 410 415

40 Met Gly Lys Glu Val Gly Asn Leu Leu Leu Glu Asn Ser Gln Leu Leu
420 425 430

45 Glu Thr Lys Asn Ala Leu Asn Val Val Lys Asn Asp Leu Ile Ala Lys
50 435 440 445

55 Val Asp Gln Leu Ser Gly Glu Gln Glu Val Leu Lys Gly Glu Leu Glu

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	450	455	460	
5				
	Ala Ala Lys Gln Ala Lys Val Lys Leu Glu Asn Arg Ile Lys Glu Leu			
10	465	470	475	480
	Glu Glu Glu Leu Lys Arg Val Lys Ser Glu Ala Val Thr Ala Arg Arg			
15		485	490	495
	Glu Pro Arg Glu Glu Val Glu Asp Val Ser Ser Tyr Leu Cys Thr Glu			
20		500	505	510
	Leu Asp Lys Ile Pro Met Ala Gln Arg Arg Arg Phe Thr Arg Val Glu			
25		515	520	525
	Met Ala Arg Val Leu Met Glu Arg Asn Gln Tyr Lys Glu Arg Leu Met			
30		530	535	540
	Glu Leu Gln Glu Ala Val Arg Trp Thr Glu Met Ile Arg Ala Ser Arg			
35		545	550	555
	Glu His Pro Ser Val Gln Glu Lys Lys Lys Ser Thr Ile Trp Gln Phe			
40		565	570	575
	Phe Ser Arg Leu Phe Ser Ser Ser Ser Ser Pro Pro Pro Ala Lys Arg			
45		580	585	590
50				
55				

EP 1 134 230 A1

5	Ser Tyr Pro Ser Val Asn Ile His Tyr Lys Ser Pro Thr Ala Ala Gly	595	600	605
10	Phe Ser Gln Arg Arg Ser His Ala Leu Cys Gln Ile Ser Ala Gly Ser	610	615	620
15	Arg Pro Leu Glu Phe Phe Pro Asp Asp Asp Cys Thr Ser Ser Ala Arg	625	630	635
20	Arg Glu Gln Lys Arg Glu Gln Tyr Arg Gln Val Arg Glu His Val Arg	645	650	655
25	Asn Asp Asp Gly Arg Leu Gln Ala Cys Gly Trp Ser Leu Pro Ala Lys	660	665	670
30	Tyr Lys Gln Leu Ser Pro Asn Gly Gly Gln Glu Asp Thr Arg Met Lys	675	680	685
35	Asn Val Pro Val Pro Val Tyr Cys Arg Pro Leu Val Glu Lys Asp Pro	690	695	700
40	Ser Thr Lys Leu Trp Cys Ala Ala Gly Val Asn Leu Ser Gly Trp Lys	705	710	715
45	Pro His Glu Glu Asp Ser Ser Asn Gly Pro Lys Pro Val Pro Gly Arg	725	730	735
50				
55				

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5

Asp Pro Leu Thr Cys Asp Arg Glu Gly Glu Gly Glu Pro Lys Ser Thr

740

745

750

10

His Pro Ser Pro Glu Lys Lys Lys Ala Lys Glu Thr Pro Glu Ala Asp

755

760

765

15

Ala Thr Ser Ser Arg Val Trp Ile Leu Thr Ser Thr Leu Thr Thr Ser

770

775

780

20

Lys Val Val Ile Ile Asp Ala Asn Gln Pro Gly Thr Ile Val Asp Gln

785

790

795

800

25

Phe Thr Val Cys Asn Ala His Val Leu Cys Ile Ser Ser Ile Pro Ala

805

810

815

30

Ala Ser Asp Ser Asp Tyr Pro Pro Gly Glu Met Phe Leu Asp Ser Asp

820

825

830

35

Val Asn Pro Glu Asp Ser Gly Ala Asp Gly Val Leu Ala Gly Ile Thr

835

840

845

40

Leu Val Gly Cys Ala Thr Arg Cys Asn Val Pro Arg Ser Asn Cys Ser

850

855

860

50

Ser Arg Gly Asp Thr Pro Val Leu Asp Lys Gly Gln Gly Asp Val Ala

55

EP 1 134 230 A1

5	865	870	875	880
	Thr Thr Ala Asn Gly Lys Val Asn Pro Ser Gln Ser Thr Glu Glu Ala			
10		885	890	895
	Thr Glu Ala Thr Glu Val Pro Asp Pro Gly Pro Ser Glu Ser Glu Ala			
15		900	905	910
	Thr Thr Val Arg Pro Gly Pro Leu Thr Glu His Val Phe Thr Asp Pro			
20		915	920	925
	Ala Pro Thr Pro Ser Ser Ser Thr Gln Pro Ala Ser Glu Asn Gly Ser			
25		930	935	940
	Glu Ser Asn Gly Thr Ile Val Gln Pro Gln Val Glu Pro Ser Gly Glu			
30		945	950	955
	Leu Ser Thr Thr Thr Ser Ser Ala Ala Pro Thr Met Trp Leu Gly Ala			
35		965	970	975
	Gln Asn Gly Trp Leu Tyr Val His Ser Ala Val Ala Asn Trp Lys Lys			
40		980	985	990
	Cys Leu His Ser Ile Lys Leu Lys Asp Ser Val Leu Ser Leu Val His			
45		995	1000	1005
50				
55				

EP 1 134 230 A1

Val Lys Gly Arg Val Leu Val Ala Leu Ala Asp Gly Thr Leu Ala Ile
5 1010 1015 1020

Phe His Arg Gly Glu Asp Gly Gln Trp Asp Leu Ser Asn Tyr His Leu
10 025 1030 1035 1040

Met Asp Leu Gly His Pro His His Ser Ile Arg Cys Met Ala Val Val
15 1045 1050 1055

Asn Asp Arg Val Trp Cys Gly Tyr Lys Asn Lys Val His Val Ile Gln
20 1060 1065 1070

Pro Lys Thr Met Gln Ile Glu Lys Ser Phe Asp Ala His Pro Arg Arg
25 1075 1080 1085

Glu Ser Gln Val Arg Gln Leu Ala Trp Ile Gly Asp Gly Val Trp Val
30 1090 1095 1100

Ser Ile Arg Leu Asp Ser Thr Leu Arg Leu Tyr His Ala His Thr His
35 1105 1110 1115 1120

Gln His Leu Gln Asp Val Asp Ile Glu Pro Tyr Val Ser Lys Met Leu
40 1125 1130 1135

Gly Thr Gly Lys Leu Gly Phe Ser Phe Val Arg Ile Thr Ala Leu Leu
45 1140 1145 1150

50
55

EP 1 134 230 A1

5 Ile Ala Gly Asn Arg Leu Trp Val Gly Thr Gly Asn Gly Val Val Ile
1155 1160 1165

10 Ser Ile Pro Leu Thr Glu Thr Val Val Leu His Arg Gly Gln Leu Leu
1170 1175 1180

15 Gly Leu Arg Ala Asn Lys Thr Ser Pro Thr Ser Gly Glu Gly Thr Arg
20 185 1190 1195 1200

25 Pro Gly Gly Ile Ile His Val Tyr Gly Asp Asp Ser Ser Asp Lys Ala
1205 1210 1215

30 Ala Ser Ser Phe Ile Pro Tyr Cys Ser Met Ala Gln Ala Gln Leu Cys
1220 1225 1230

35 Phe His Gly His Arg Asp Ala Val Lys Phe Phe Val Ser Val Pro Gly
1235 1240 1245

40 Asn Val Leu Ala Thr Leu Asn Gly Ser Val Leu Asp Ser Pro Ser Glu
1250 1255 1260

45 Gly Pro Gly Pro Ala Ala Pro Ala Ala Asp Ala Glu Gly Gln Lys Leu
50 265 1270 1275 1280

55 Lys Asn Ala Leu Val Leu Ser Gly Gly Glu Gly Tyr Ile Asp Phe Arg

EP 1 134 230 A1

	1285	1290	1295
5			
	Ile Gly Asp Gly Glu Asp Asp Glu Thr Glu Glu Cys Ala Gly Asp Val		
10	1300	1305	1310
	Asn Gln Thr Lys Pro Ser Leu Ser Lys Ala Glu Arg Ser His Ile Ile		
15	1315	1320	1325
	Val Trp Gln Val Ser Tyr Thr Pro Glu		
20	1330	1335	
25			
	<210> 12		
30	<211> 1336		
	<212> PRT		
	<213> Mouse		
35			
	<400> 12		
40	Met Met Glu Ile Gln Met Asp Glu Gly Gly Gly Val Val Val Tyr Gln		
	1	5	10 15
45	Asp Asp Tyr Cys Ser Gly Ser Val Met Ser Glu Arg Val Ser Gly Leu		
	20	25	30
50	Ala Gly Ser Ile Tyr Arg Glu Phe Glu Arg Leu Ile His Cys Tyr Asp		
	35	40	45
55			

EP 1 134 230 A1

5 Glu Glu Val Val Lys Glu Leu Met Pro Leu Val Val Asn Val Leu Glu
50 55 60

10 Asn Leu Asp Ser Val Leu Ser Glu Asn Gln Glu His Glu Val Glu Leu
65 70 75 80

15 Glu Leu Leu Arg Glu Asp Asn Glu Gln Leu Leu Thr Gln Tyr Glu Arg
20 85 90 95

25 Glu Lys Ala Leu Arg Lys Gln Ala Glu Glu Lys Phe Ile Glu Phe Glu
100 105 110

30 Asp Ala Leu Glu Gln Glu Lys Lys Glu Leu Gln Ile Gln Val Glu His
115 120 125

35 Tyr Glu Phe Gln Thr Arg Gln Leu Glu Leu Lys Ala Lys Asn Tyr Ala
130 135 140

40 Asp Gln Ile Ser Arg Leu Glu Glu Arg Glu Ser Glu Met Lys Lys Glu
45 145 150 155 160

50 Tyr Asn Ala Leu His Gln Arg His Thr Glu Met Ile Gln Thr Tyr Val
165 170 175

55 Glu His Ile Glu Arg Ser Lys Met Gln Gln Val Gly Gly Ser Gly Gln

EP 1 134 230 A1

5		180		185		190
	Thr	Glu	Ser	Ser	Leu	Pro
	Gly	Arg	Arg	Lys	Glu	Arg
	Pro	Thr	Ser	Leu		
10		195		200		205
	Asn	Val	Phe	Pro	Leu	Ala
	Asp	Gly	Met	Val	Arg	Ala
	Gln	Met	Gly	Gly		
15		210		215		220
	Lys	Leu	Val	Pro	Ala	Gly
	Asp	His	Trp	His	Leu	Ser
	Asp	Leu	Gly	Gln		
20		225		230		235
						240
25	Leu	Gln	Ser	Ser	Ser	Tyr
	Gln	Cys	Pro	Asn	Asp	Glu
	Met	Ser	Glu			
30		245		250		255
	Ser	Gly	Gln	Ser	Ser	Ala
	Ala	Ala	Ala	Thr	Pro	Ser
	Thr	Thr	Gly	Thr	Lys	
35		260		265		270
	Ser	Asn	Thr	Pro	Thr	Ser
	Ser	Val	Pro	Ser	Ala	Ala
	Val	Thr	Pro	Leu		
40		275		280		285
	Asn	Glu	Ser	Leu	Gln	Pro
	Leu	Gly	Asp	Tyr	Val	Ser
	Val	Thr	Lys	Asn		
45		290		295		300
	Asn	Lys	Gln	Ala	Arg	Glu
	Lys	Arg	Asn	Ser	Arg	Asn
	Met	Glu	Val	Gln		
50		305		310		315
						320
55						

EP 1 134 230 A1

5 Val Thr Gln Glu Met Arg Asn Val Ser Ile Gly Met Gly Ser Ser Asp
325 330 335

10 Glu Trp Ser Asp Val Gln Asp Ile Ile Asp Ser Thr Pro Glu Leu Asp
340 345 350

15 Val Cys Pro Glu Thr Arg Leu Glu Arg Thr Gly Ser Ser Pro Thr Gln
355 360 365

20 Gly Ile Val Asn Lys Ala Phe Gly Ile Asn Thr Asp Ser Leu Tyr His
370 375 380

30 Glu Leu Ser Thr Ala Gly Ser Glu Val Ile Gly Asp Val Asp Glu Gly
385 390 395 400

35 Ala Asp Leu Leu Gly Glu Phe Ser Val Arg Asp Asp Phe Phe Gly Met
405 410 415

40 Gly Lys Glu Val Gly Asn Leu Leu Leu Glu Asn Ser Gln Leu Leu Glu
420 425 430

45 Thr Lys Asn Ala Leu Asn Val Val Lys Asn Asp Leu Ile Ala Lys Val
435 440 445

50 Asp Gln Leu Ser Gly Glu Gln Glu Val Leu Lys Gly Glu Leu Glu Ala
450 455 460

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5

Ala Lys Gln Ala Lys Val Lys Leu Glu Asn Arg Ile Lys Glu Leu Glu

10

465 470 475 480

15

Glu Glu Leu Lys Arg Val Lys Ser Glu Ala Val Thr Ala Arg Arg Glu

485 490 495

20

Pro Arg Glu Glu Val Glu Asp Val Ser Ser Tyr Leu Cys Thr Glu Leu

500 505 510

25

Asp Lys Ile Pro Met Ala Gln Arg Arg Arg Phe Thr Arg Val Glu Met

515 520 525

30

Ala Arg Val Leu Met Glu Arg Asn Gln Tyr Lys Glu Arg Leu Met Glu

530 535 540

35

Leu Gln Glu Ala Val Arg Trp Thr Glu Met Ile Arg Ala Ser Arg Glu

40

545 550 555 560

45

His Pro Ser Val Gln Glu Lys Lys Lys Ser Thr Ile Trp Gln Phe Phe

565 570 575

50

Ser Arg Leu Phe Ser Ser Ser Ser Ser Pro Pro Pro Ala Lys Arg Ser

580 585 590

55

Tyr Pro Ser Val Asn Ile His Tyr Lys Ser Pro Thr Ala Ala Gly Phe

EP 1 134 230 A1

5	595	600	605
10	Ser Gln Arg Arg Ser His Ala Leu Cys Gln Ile Ser Ala Gly Ser Arg		
	610	615	620
15	Pro Leu Glu Phe Phe Pro Asp Asp Asp Cys Thr Ser Ser Ala Arg Arg		
	625	630	635 640
20	Glu Gln Lys Arg Glu Gln Tyr Arg Gln Val Arg Glu His Val Arg Asn		
	645	650	655
25	Asp Asp Gly Arg Leu Gln Ala Cys Gly Trp Ser Leu Pro Ala Lys Tyr		
30	660	665	670
35	Lys Gln Leu Ser Pro Asn Gly Gly Gln Glu Asp Thr Arg Met Lys Asn		
	675	680	685
40	Val Pro Val Pro Val Tyr Cys Arg Pro Leu Val Glu Lys Asp Pro Ser		
	690	695	700
45	Thr Lys Leu Trp Cys Ala Ala Gly Val Asn Leu Ser Gly Trp Lys Pro		
	705	710	715 720
50	His Glu Glu Asp Ser Ser Asn Gly Pro Lys Pro Val Pro Gly Arg Asp		
	725	730	735

55

EP 1 134 230 A1

5 Pro Leu Thr Cys Asp Arg Glu Gly Glu Gly Glu Pro Lys Ser Thr His
740 745 750

10 Pro Ser Pro Glu Lys Lys Lys Ala Lys Glu Thr Pro Glu Ala Asp Ala
755 760 765

15 Thr Ser Ser Arg Val Trp Ile Leu Thr Ser Thr Leu Thr Thr Ser Lys
770 775 780

20 Val Val Ile Ile Asp Ala Asn Gln Pro Gly Thr Ile Val Asp Gln Phe
25 785 790 795 800

30 Thr Val Cys Asn Ala His Val Leu Cys Ile Ser Ser Ile Pro Ala Ala
805 810 815

35 Ser Asp Ser Asp Tyr Pro Pro Gly Glu Met Phe Leu Asp Ser Asp Val
820 825 830

40 Asn Pro Glu Asp Ser Gly Ala Asp Gly Val Leu Ala Gly Ile Thr Leu
835 840 845

45 Val Gly Cys Ala Thr Arg Cys Asn Val Pro Arg Ser Asn Cys Ser Ser
50 850 855 860

55 Arg Gly Asp Thr Pro Val Leu Asp Lys Gly Gln Gly Asp Val Ala Thr
865 870 875 880

5

Thr Ala Asn Gly Lys Val Asn Pro Ser Gln Ser Thr Glu Glu Ala Thr

10

885

890

895

15

Glu Ala Thr Glu Val Pro Asp Pro Gly Pro Ser Glu Ser Glu Ala Thr

900

905

910

20

Thr Val Arg Pro Gly Pro Leu Thr Glu His Val Phe Thr Asp Pro Ala

915

920

925

25

Pro Thr Pro Ser Ser Ser Thr Gln Pro Ala Ser Glu Asn Gly Ser Glu

930

935

940

30

Ser Asn Gly Thr Ile Val Gln Pro Gln Val Glu Pro Ser Gly Glu Leu

945

950

955

960

35

Ser Thr Thr Thr Ser Ser Ala Ala Pro Thr Met Trp Leu Gly Ala Gln

965

970

975

40

Asn Gly Trp Leu Tyr Val His Ser Ala Val Ala Asn Trp Lys Lys Cys

45

980

985

990

50

Leu His Ser Ile Lys Leu Lys Asp Ser Val Leu Ser Leu Val His Val

995

1000

1005

55

Lys Gly Arg Val Leu Val Ala Leu Ala Asp Gly Thr Leu Ala Ile Phe

EP 1 134 230 A1

5	1010	1015	1020	
	His Arg Gly Glu Asp Gly Gln Trp Asp Leu Ser Asn Tyr His Leu Met			
10	025	1030	1035	1040
	Asp Leu Gly His Pro His His Ser Ile Arg Cys Met Ala Val Val Asn			
15		1045	1050	1055
	Asp Arg Val Trp Cys Gly Tyr Lys Asn Lys Val His Val Ile Gln Pro			
20		1060	1065	1070
	Lys Thr Met Gln Ile Glu Lys Ser Phe Asp Ala His Pro Arg Arg Glu			
25		1075	1080	1085
	Ser Gln Val Arg Gln Leu Ala Trp Ile Gly Asp Gly Val Trp Val Ser			
30		1090	1095	1100
	Ile Arg Leu Asp Ser Thr Leu Arg Leu Tyr His Ala His Thr His Gln			
35		1105	1110	1115
	His Leu Gln Asp Val Asp Ile Glu Pro Tyr Val Ser Lys Met Leu Gly			
40		1125	1130	1135
	Thr Gly Lys Leu Gly Phe Ser Phe Val Arg Ile Thr Ala Leu Leu Ile			
45		1140	1145	1150
50				
55				

EP 1 134 230 A1

5 Ala Gly Asn Arg Leu Trp Val Gly Thr Gly Asn Gly Val Val Ile Ser
1155 1160 1165

10 Ile Pro Leu Thr Glu Thr Val Val Leu His Arg Gly Gln Leu Leu Gly
1170 1175 1180

15 Leu Arg Ala Asn Lys Thr Ser Pro Thr Ser Gly Glu Gly Thr Arg Pro
185 1190 1195 1200

20 Gly Gly Ile Ile His Val Tyr Gly Asp Asp Ser Ser Asp Lys Ala Ala
25 1205 1210 1215

30 Ser Ser Phe Ile Pro Tyr Cys Ser Met Ala Gln Ala Gln Leu Cys Phe
1220 1225 1230

35 His Gly His Arg Asp Ala Val Lys Phe Phe Val Ser Val Pro Gly Asn
1235 1240 1245

40 Val Leu Ala Thr Leu Asn Gly Ser Val Leu Asp Ser Pro Ser Glu Gly
1250 1255 1260

45 Pro Gly Pro Ala Ala Pro Ala Ala Asp Ala Glu Gly Gln Lys Leu Lys
265 1270 1275 1280

50 Asn Ala Leu Val Leu Ser Gly Gly Glu Gly Tyr Ile Asp Phe Arg Ile
55 1285 1290 1295

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5

Gly Asp Gly Glu Asp Asp Glu Thr Glu Glu Cys Ala Gly Asp Val Asn
 1300 1305 1310

10

Gln Thr Lys Pro Ser Leu Ser Lys Ala Glu Arg Ser His Ile Ile Val
 1315 1320 1325

15

Trp Gln Val Ser Tyr Thr Pro Glu
 1330 1335

20

25

<210> 13

<211> 430

30

<212> PRT

<213> Mouse

35

<400> 13

Met Ser Gly Val Arg Pro Pro Ile Met Asn Gly Pro Met His Pro Arg
 1 5 10 15

40

Pro Leu Val Ala Leu Leu Asp Gly Arg Asp Cys Thr Val Glu Met Pro
 20 25 30

45

Ile Leu Lys Asp Val Ala Thr Val Ala Phe Cys Asp Ala Gln Ser Thr
 35 40 45

50

55

EP 1 134 230 A1

5 Gln Glu Ile His Glu Lys Val Leu Asn Glu Ala Val Gly Ala Leu Met
50 55 60

10 Tyr His Thr Ile Thr Leu Thr Arg Glu Asp Leu Glu Lys Phe Lys Ala
65 70 75 80

15 Leu Arg Ile Ile Val Arg Ile Gly Ser Gly Phe Asp Asn Ile Asp Ile
85 90 95

20 Lys Ser Ala Gly Asp Leu Gly Ile Ala Val Cys Asn Val Pro Ala Ala
25 100 105 110

30 Ser Val Glu Glu Thr Ala Asp Ser Thr Leu Cys His Ile Leu Asn Leu
115 120 125

35 Tyr Arg Arg Thr Thr Trp Leu His Gln Ala Leu Arg Glu Gly Thr Arg
130 135 140

40 Val Gln Ser Val Glu Gln Ile Arg Glu Val Ala Ser Gly Ala Ala Arg
145 150 155 160

45 Ile Arg Gly Glu Thr Leu Gly Ile Ile Gly Leu Gly Arg Val Gly Gln
165 170 175

50 Ala Val Ala Leu Arg Ala Lys Ala Phe Gly Phe Asn Val Leu Phe Tyr
55 180 185 190

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5

Asp Pro Tyr Leu Ser Asp Gly Ile Glu Arg Ala Leu Gly Leu Gln Arg

195 200 205

10

Val Ser Thr Leu Gln Asp Leu Leu Phe His Ser Asp Cys Val Thr Leu

210 215 220

15

His Cys Gly Leu Asn Glu His Asn His His Leu Ile Asn Asp Phe Thr

225 230 235 240

20

Val Lys Gln Met Arg Gln Gly Ala Phe Leu Val Asn Thr Ala Arg Gly

245 250 255

25

Gly Leu Val Asp Glu Lys Ala Val Ala Gln Ala Leu Lys Glu Gly Arg

260 265 270

30

Ile Arg Gly Ala Ala Leu Asp Val His Glu Ser Glu Pro Phe Ser Phe

275 280 285

35

Ser Gln Gly Pro Leu Lys Asp Ala Pro Asn Leu Ile Cys Thr Pro His

290 295 300

40

Ala Ala Trp Tyr Ser Glu Gln Ala Ser Ile Glu Met Arg Glu Glu Ala

305 310 315 320

45

Ala Arg Glu Ile Arg Arg Ala Ile Thr Gly Arg Ile Pro Asp Ser Leu

50

EP 1 134 230 A1

5		325		330		335
	Lys Asn Cys Val Asn Lys Asp His Leu Thr Ala Ala Thr His Trp Ala					
10		340		345		350
	Ser Met Asp Pro Ala Val Val His Pro Glu Leu Asn Gly Ala Ala Tyr					
15		355		360		365
	Ser Arg Tyr Pro Pro Gly Val Val Ser Val Ala Pro Thr Gly Ile Pro					
20		370		375		380
	Ala Ala Val Glu Gly Ile Val Pro Ser Ala Met Ser Leu Ser His Gly					
25		385		390		395
						400
30	Leu Pro Pro Val Ala His Pro Pro His Ala Pro Ser Pro Gly Gln Thr					
			405		410	415
35	Val Lys Pro Glu Ala Asp Arg Asp His Thr Ser Asp Gln Leu					
40		420		425		430
45	<210> 14					
	<211> 1508					
50	<212> PRT					
	<213> Mouse					
55						

EP 1 134 230 A1

<400> 14

Met Met Ala Gly Glu Gly Ser Thr Ile Thr Ser Arg Ile Lys Asn Leu

1 5 10 15

Leu Arg Ser Pro Ser Ile Lys Leu Arg Arg Ser Lys Ala Gly Asn Arg

20 25 30

Arg Glu Asp Leu Ser Ser Lys Val Thr Leu Glu Lys Val Leu Gly Val

35 40 45

Thr Val Ser Gly Gly Arg Gly Leu Ala Cys Glu Pro Arg Ser Gly Leu

50 55 60

Val Ala Tyr Pro Ala Gly Cys Val Val Val Leu Phe Asn Pro Arg Lys

65 70 75 80

His Lys Gln His His Ile Leu Asn Ser Ser Arg Lys Thr Ile Thr Ala

85 90 95

Leu Ala Phe Ser Pro Asp Gly Lys Tyr Leu Val Thr Gly Glu Ser Gly

100 105 110

His Met Pro Ala Val Arg Val Trp Asp Val Ala Glu Arg Ser Gln Val

115 120 125

Ala Glu Leu Gln Glu His Lys Tyr Gly Val Ala Cys Val Ala Phe Ser

EP 1 134 230 A1

	130	135	140	
5				
	Pro Ser Ala Lys Tyr Ile Val Ser Val Gly Tyr Gln His Asp Met Ile			
10	145	150	155	160
	Val Asn Val Trp Ala Trp Lys Lys Asn Ile Val Val Ala Ser Asn Lys			
15		165	170	175
	Val Ser Ser Arg Val Thr Ala Val Ser Phe Ser Glu Asp Cys Ser Tyr			
20		180	185	190
	Phe Val Thr Ala Gly Asn Arg His Ile Lys Phe Trp Tyr Leu Asp Asp			
25		195	200	205
	Ser Lys Thr Ser Lys Val Asn Ala Thr Val Pro Leu Leu Gly Arg Ser			
30		210	215	220
	Gly Leu Leu Gly Glu Leu Arg Asn Asn Leu Phe Thr Asp Val Ala Cys			
35		225	230	235
	Gly Arg Gly Glu Lys Ala Asp Ser Thr Phe Cys Ile Thr Ser Ser Gly			
40		245	250	255
	Leu Leu Cys Glu Phe Ser Asp Arg Arg Leu Leu Asp Lys Trp Val Glu			
45		260	265	270
50				
55				

EP 1 134 230 A1

5 Leu Arg Asn Thr Asp Ser Phe Thr Thr Thr Val Ala His Cys Ile Ser
275 280 285

10 Val Thr Gln Glu Tyr Ile Phe Cys Gly Cys Ala Asp Gly Thr Val Arg
290 295 300

15 Leu Phe Asn Pro Ser Asn Leu His Phe Leu Ser Thr Leu Pro Arg Pro
305 310 315 320

20 His Ala Leu Gly Thr Asp Ile Ala Ser Ile Thr Glu Ala Ser Arg Leu
325 330 335

25 Phe Ser Gly Gly Val Asn Ala Arg Tyr Pro Asp Thr Ile Ala Leu Thr
340 345 350

30 Phe Asp Pro Thr Asn Gln Trp Leu Ser Cys Val Tyr Asn Asp His Ser
355 360 365

35 Ile Tyr Val Trp Asp Val Arg Asp Pro Lys Lys Val Gly Lys Val Tyr
370 375 380

40 Ser Ala Leu Tyr His Ser Ser Cys Val Trp Ser Val Glu Val Tyr Pro
385 390 395 400

45 Glu Ile Lys Asp Ser His Gln Ala Cys Leu Pro Leu Ser Ser Phe Ile
405 410 415

50

55

EP 1 134 230 A1

5

Thr Cys Ser Ser Asp Asn Thr Ile Arg Leu Trp Asn Thr Glu Ser Ser

10

420

425

430

Gly Val His Gly Ser Thr Leu His Arg Asn Ile Leu Ser Asn Asp Leu

15

435

440

445

Ile Lys Ile Ile Tyr Val Asp Gly Asn Thr Gln Ala Leu Leu Asp Thr

20

450

455

460

25

Glu Leu Pro Gly Gly Asp Lys Ala Asp Gly Ser Leu Met Asp Pro Arg

465

470

475

480

30

Val Gly Ile Arg Ser Val Cys Ile Ser Pro Asn Gly Gln His Leu Ala

485

490

495

35

Ser Gly Asp Arg Met Gly Thr Leu Arg Ile His Glu Leu Gln Ser Leu

40

500

505

510

Ser Glu Met Leu Lys Val Glu Ala His Asp Ser Glu Ile Leu Cys Leu

45

515

520

525

Glu Tyr Ser Lys Pro Asp Thr Gly Leu Lys Leu Leu Ala Ser Ala Ser

50

530

535

540

55

Arg Asp Arg Leu Ile His Glu Leu Asp Ala Gly Arg Glu Tyr Ser Leu

EP 1 134 230 A1

5	545	550	555	560
	Gln Gln Thr Leu Asp Glu His Ser Ser Ser Ile Thr Ala Val Lys Phe			
10		565	570	575
	Ala Ala Ser Asp Gly Gln Val Arg Met Ile Ser Cys Gly Ala Asp Lys			
15		580	585	590
	Ser Ile Tyr Phe Arg Thr Ala Gln Lys Ser Gly Glu Gly Val Gln Phe			
20		595	600	605
	Thr Arg Thr His His Val Val Arg Lys Thr Thr Leu Tyr Asp Met Asp			
25		610	615	620
	Val Glu Pro Ser Trp Lys Tyr Thr Ala Ile Gly Cys Gln Asp Arg Asn			
30		625	630	635
				640
35				
	Ile Arg Ile Phe Asn Ile Ser Ser Gly Lys Gln Lys Lys Leu Phe Lys			
40		645	650	655
	Gly Ser Gln Gly Glu Asp Gly Thr Leu Ile Lys Val Gln Thr Asp Pro			
45		660	665	670
	Ser Gly Ile Tyr Ile Ala Thr Ser Cys Ser Asp Lys Asn Leu Ser Ile			
50		675	680	685
55				

EP 1 134 230 A1

5 Phe Asp Phe Ser Ser Gly Glu Cys Val Ala Thr Met Phe Gly His Ser
 690 695 700
 10 Glu Ile Val Thr Gly Met Lys Phe Ser Asn Asp Cys Lys His Leu Ile
 705 710 715 720
 15 Ser Val Ser Gly Asp Ser Cys Ile Phe Val Trp Arg Leu Ser Ser Glu
 725 730 735
 20 Met Thr Ile Ser Met Arg Gln Arg Leu Arg Glu Arg Arg Gln Arg Gln
 25 740 745 750
 30 Arg Gly Ile Lys Gln Gln Gly Pro Thr Ser Pro Gln Arg Ala Ser Gly
 755 760 765
 35 Ala Lys Gln His His Ala Pro Val Val Pro Pro Ser Gly Pro Ala Leu
 770 775 780
 40 Ser Ser Asp Ser Asp Lys Glu Gly Glu Asp Glu Gly Thr Glu Glu Glu
 785 790 795 800
 45 Glu Leu Pro Ala Leu Pro Ile Leu Ser Lys Ser Thr Lys Lys Glu Leu
 805 810 815
 50 Ala Ser Gly Ser Ser Pro Ala Leu Leu Arg Ser Leu Ser His Trp Glu
 55 820 825 830

EP 1 134 230 A1

5

Met Ser Arg Ala Gln Glu Thr Met Glu Tyr Leu Asp Pro Ala Pro Val

10

835

840

845

15

Ala Asn Thr Gly Pro Lys Arg Arg Gly Arg Trp Ala Gln Pro Gly Val

850

855

860

20

Glu Leu Ser Val Arg Ser Met Leu Asp Leu Arg Gln Ile Glu Thr Leu

865

870

875

880

25

Ala Pro Ser Pro Arg Gly Pro Ser Gln Asp Ser Leu Ala Val Ser Pro

885

890

895

30

Ala Gly Pro Gly Lys His Gly Pro Gln Ala Pro Glu Leu Ser Cys Val

900

905

910

35

Ser Gln Asn Glu Arg Ala Pro Arg Leu Gln Thr Ser Gln Pro Cys Ser

915

920

925

40

Cys Pro Asp Ile Ile Gln Leu Leu Ser Gln Glu Glu Gly Val Phe Ala

45

930

935

940

50

Gln Asp Leu Glu Pro Ala Pro Ile Glu Asp Gly Ile Val Tyr Pro Glu

945

950

955

960

55

Pro Ser Asp Ser Pro Thr Met Asp Thr Ser Ala Phe Gln Val Gln Ala

EP 1 134 230 A1

5	965	970	975
10	Pro Thr Gly Gly Ser Leu Gly Arg Met Tyr Pro Gly Ser Arg Gly Ser		
	980	985	990
15	Glu Lys His Ser Pro Asp Ser Ala Cys Ser Val Asp Tyr Ser Ser Ser		
	995	1000	1005
20	Arg Leu Ser Ser Pro Glu His Pro Asn Glu Asp Ser Glu Ser Thr Glu		
	1010	1015	1020
25	Pro Leu Ser Val Asp Gly Ile Ser Ser Asp Leu Glu Glu Pro Ala Glu		
30	025	1030	1035 1040
35	Gly Asp Glu Asp Glu Glu Glu Glu Gly Gly Thr Gly Leu Cys Gly Leu		
	1045	1050	1055
40	Gln Glu Gly Gly Pro Arg Thr Pro Asp Gln Glu Gln Phe Leu Lys Gln		
	1060	1065	1070
45	Leu Phe Glu Thr Leu Ala Asn Gly Thr Ala Pro Gly Gly Pro Ala Arg		
	1075	1080	1085
50	Val Leu Glu Arg Thr Glu Ser Arg Ser Ile Ser Ser Arg Phe Leu Leu		
	1090	1095	1100
55			

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5 Gln Val Gln Thr Leu Pro Leu Arg Glu Pro Ser Leu Ser Ser Ser Gly
105 1110 1115 1120

10 Leu Ala Leu Thr Ser Arg Pro Asp Gln Val Ser Gln Val Ser Gly Glu
1125 1130 1135

15 Gln Leu Lys Gly Ser Gly Ala Thr Pro Pro Gly Ala Pro Pro Glu Met
1140 1145 1150

20 Glu Pro Ser Ser Gly Asn Ser Gly Pro Lys Gln Val Ala Pro Val Leu
1155 1160 1165

25 Leu Thr Arg Arg Arg Asn Asn Leu Asp Asn Ser Trp Ala Ser Lys Lys
1170 1175 1180

30 Met Ala Ala Thr Arg Pro Leu Ala Gly Leu Gln Lys Ala Gln Ser Val
185 1190 1195 1200

35 His Ser Leu Val Pro Gln Asp Glu Val Pro Ser Ser Arg Pro Leu Leu
1205 1210 1215

40 Phe Arg Glu Ala Glu Thr Gln Gly Ser Leu Gly Ser Leu Pro Gln Ala
1220 1225 1230

45 Gly Gly Cys Ser Ser Gln Pro His Ser Tyr Gln Asn His Thr Thr Ser
1235 1240 1245

50

55

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5

Ser Met Ala Lys Leu Ala Arg Ser Ile Ser Val Gly Glu Asn Pro Gly

10

1250 1255 1260

Leu Ala Thr Glu Pro Gln Ala Pro Ala Pro Ile Arg Ile Ser Pro Phe

15

265 1270 1275 1280

Asn Lys Leu Ala Leu Pro Ser Arg Ala His Leu Val Leu Asp Ile Pro

20

1285 1290 1295

Lys Pro Leu Pro Asp Arg Pro Thr Leu Thr Thr Phe Ser Pro Val Ser

25

1300 1305 1310

Lys Gly Leu Thr His Asn Glu Thr Glu Gln Ser Gly Pro Leu Arg Glu

30

1315 1320 1325

Pro Arg Lys Ala His Thr Thr Val Glu Lys His Ser Cys Leu Gly Glu

35

1330 1335 1340

Gly Thr Thr His Lys Ser Arg Thr Glu Cys Gln Ala Tyr Pro Gly Pro

40

345 1350 1355 1360

Asn His Pro Cys Arg Gln Gln Leu Pro Val Asn Asn Leu Leu Gln Ala

50

1365 1370 1375

Glu Ser Leu Gln Pro Leu Ser Pro Glu Lys Thr Arg Asn Pro Val Glu

55

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5	1380	1385	1390
	Ser Ser Arg Pro Gly Val Ala Leu Ser Gln Asp Ser Glu Leu Ala Leu		
10	1395	1400	1405
	Ser Leu Gln Gln Cys Glu Gln Leu Val Ala Glu Leu Gln Gly Asn Val		
15	1410	1415	1420
	Arg Gln Ala Val Glu Leu Tyr Arg Ala Val Thr Ser Cys Lys Thr Pro		
20	425	1430	1435 1440
	Ser Ala Glu Gln Ser His Ile Thr Arg Leu Leu Arg Asp Thr Phe Ser		
25	1445	1450	1455
30	Pro Val Arg Gln Glu Leu Glu Val Leu Ala Gly Ala Val Leu Ser Ser		
35	1460	1465	1470
	Pro Gly Gly Ser Pro Gly Ala Val Ala Ala Glu Gln Thr Gln Ala Leu		
40	1475	1480	1485
	Leu Glu Gln Tyr Ser Glu Leu Leu Leu Arg Ala Val Glu Arg Arg Met		
45	1490	1495	1500
50	Glu Arg Arg Leu		
55	505		

5

<210> 15

10

<211> 244

<212> PRT

15

<213> Mouse

<400> 15

20

Ser Ser Lys Tyr Ser Asn Glu Ser Arg Ser Gln Ala Asp Ser Gly Phe

1 5 10 15

25

Leu Gly Leu Arg Pro Thr Ser Val Asp Pro Ala Leu Arg Arg Arg Arg

20 25 30

30

Arg Gly Pro Arg Asn Lys Lys Arg Gly Trp Arg Arg Leu Ala Glu Glu

35 40 45

35

Pro Leu Gly Leu Glu Val Asp Gln Phe Leu Glu Asp Val Arg Leu Gln

50 55 60

40

Glu Arg Thr Thr Gly Gly Leu Leu Ala Glu Ala Pro Asn Glu Lys Leu

45

65 70 75 80

50

Phe Phe Val Asp Thr Gly Phe Lys Arg Lys Glu Pro Arg Lys Lys Arg

85 90 95

55

Thr Leu Val Gln Lys Lys Ser Gln Arg Leu Gln Lys Pro Leu Arg Val

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5	100	105	110
10	Asp Leu Ala Leu Glu Asn His Ser Lys Ile Pro Ala Pro Lys Asp Ile		
	115	120	125
15	Leu Ala His Gln Val Pro Asn Ala Lys Lys Leu Arg Arg Lys Glu Glu		
	130	135	140
20	Leu Trp Glu Lys Leu Ala Lys Gln Gly Glu Leu Pro Arg Asp Val Arg		
	145	150	155 160
25	Lys Ala Gln Ala Arg Leu Leu Ser Pro Pro Thr Pro Lys Ala Lys Pro		
	165	170	175
30	Gly Pro Gln Asp Ile Ile Glu Arg Pro Phe Tyr Asp Leu Trp Asn Pro		
35	180	185	190
40	Asp Asn Pro Leu Asp Thr Pro Leu Ile Gly Gln Asp Ala Phe Phe Leu		
	195	200	205
45	Glu Gln Thr Lys Lys Lys Gly Val Arg Arg Pro Gln Arg Leu His Ile		
	210	215	220
50	Lys Pro Ser Gln Val Pro Ala Val Glu Val Ile Pro Ala Gly Ala Ser		
	225	230	235 240

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5 Tyr Asn Pro Thr

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 15 <212> PRT
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 1 5 10 15

25 Gln Ala Asp Ser Gly Phe Leu Gly Leu Arg Pro Thr Ser Val Asp Pro
 20 25 30

30 Ala Leu Arg Arg Arg Arg Arg Gly Pro Arg Asn Lys Lys Arg Gly Trp
 35 40 45

35 Arg Arg Leu Ala Glu Glu Pro Leu Gly Leu Glu Val Asp Gln Phe Leu
 50 55 60

40 Glu Asp Val Arg Leu Gln Glu Arg Thr Thr Gly Gly Leu Leu Ala Glu
 65 70 75 80

45 Ala Pro Asn Glu Lys Leu Phe Phe Val Asp Thr Gly Phe Lys Arg Lys
 85 90 95

Glu Pro Arg Lys Lys Arg Thr Leu Val Gln Lys Lys Ser Gln Arg Leu
 100 105 110

50 Gln Lys Pro Leu Arg Val Asp Leu Ala Leu Glu Asn His Ser Lys Ile
 115 120 125

55 Pro Ala Pro Lys Asp Ile Leu Ala His Gln Val Pro Asn Ala Lys Lys

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	130	135	140
5	Leu Arg Arg Lys Glu Glu Leu Trp Glu Lys Leu Ala Lys Gln Gly Glu		
	145	150	155 160
10	Leu Pro Arg Asp Val Arg Lys Ala Gln Ala Arg Leu Leu Ser Pro Pro		
	165	170	175
15	Thr Pro Lys Ala Lys Pro Gly Pro Gln Asp Ile Ile Glu Arg Pro Phe		
	180	185	190
20	Tyr Asp Leu Trp Asn Pro Asp Asn Pro Leu Asp Thr Pro Leu Ile Gly		
	195	200	205
25	Gln Asp Ala Phe Phe Leu Glu Gln Thr Lys Lys Lys Gly Val Arg Arg		
	210	215	220
30	Pro Gln Arg Leu His Ile Lys Pro Ser Gln Val Pro Ala Val Glu Val		
	225	230	235 240
35	Ile Pro Ala Gly Ala Ser Tyr Asn Pro Thr Phe Glu Asp His Gln Ala		
	245	250	255
40	Leu Leu Arg Glu Ala His Glu Val Glu Leu Gln Arg Glu Lys Glu Ala		
	260	265	270
45	Glu Lys Leu Glu Arg Gln Leu Ala Leu Pro Thr Ser Glu Gln Ala Ala		
	275	280	285
50	Thr Gln Glu Ser Val Phe Arg Glu Met Cys Glu Gly Leu Leu Glu Glu		
	290	295	300
55	Ser Asp Gly Glu Asp Glu His Glu Ala Gly Arg Ala Gly Gln Pro Glu		
	305	310	315 320
60	Ala Gly Asp Gly Thr Thr Glu Ile Ser Pro Thr Gly Ala Ala Gly Pro		
	325	330	335
65	Glu Lys Arg Met Glu Lys Lys Thr Glu Gln Gln Arg Arg Arg Glu Lys		

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5 340 345 350
 Ala Ala Arg Lys Leu Arg Val Gln Gln Ala Ala Leu Arg Ala Ala Arg
 355 360 365
 10 Leu Gln His Gln Glu Leu Phe Arg Leu Arg Gly Ile Lys Ala Gln Val
 370 375 380
 Ala Arg Arg Leu Ala Glu Leu Ala Arg Arg Arg Glu Gln Arg Arg Ile
 15 385 390 395 400
 Arg Arg Leu Ala Glu Ala Asp Lys Pro Arg Arg Leu Gly Arg Leu Lys
 405 410 415
 20 Tyr Gln Ala Pro Asp Ile Asp Val Gln Leu Ser Ser Glu Leu Ser Gly
 420 425 430
 25 Ser Leu Arg Thr Leu Lys Pro Glu Gly His Ile Leu Arg Asp Arg Phe
 435 440 445
 Lys Ser Phe Gln Lys Arg Asn Met Ile Glu Pro Arg Glu Arg Ala Lys
 30 450 455 460
 Phe Lys Arg Lys Tyr Lys Val Lys Leu Val Glu Lys Arg Ala Tyr Arg
 35 465 470 475 480
 Glu Ile Gln Leu

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 45 <211> 27
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15

20 <210> 18

<211> 31

<212> DNA

25

<213> Artificial Sequence

30 <220>

<223> Description of Artificial Sequence:Synthetic DNA

35

<400> 18

atgaattctc agttgttctt tgtgacactg a

31

40

Claims

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1. A polypeptide comprising an amino acid sequence selected from the amino acid sequences represented by any one of SEQ ID NOS:10 to 16.
2. A polypeptide comprising an amino acid sequence in which at least one amino acid has been deleted, substituted or added in an amino acid sequence selected from the amino acid sequences represented by any one of SEQ ID NOS:14 to 16 and is capable of binding to JNK3.
3. A DNA which encodes the polypeptide of claim 1 or 2.
- 50 4. A DNA comprising a nucleotide sequence selected from the nucleotide sequences represented by any one of SEQ ID NOS:2 to 8.
5. A DNA which hybridizes with a DNA comprising the nucleotide sequence represented by any one of SEQ ID NOS:

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6 to 8 under stringent conditions, and encodes a polypeptide capable of binding to JNK3.

6. A recombinant DNA obtained by inserting the DNA of any one of claims 3 to 5 into a vector.

7. The recombinant DNA according to claim 6, which is a recombinant DNA selected from plasmid pcDNA3-S-JSAP1b, plasmid pcDNA3-S-JSAP1c, plasmid pcDNA3-S-JSAP4, plasmid pGAD10-JSAP5, and plasmid pcDNA3-His-S-JSAP5.

8. A transformant comprising the recombinant DNA of claim 6 or 7.

9. The transformant according to claim 8, which is a transformant selected from a microorganism, an animal cell, a plant cell, and an insect cell.

10. The transformant according to claim 9, which is a microorganism belonging to the genus *Escherichia*.

11. The transformant according to claim 10, wherein the microorganism belonging to the genus *Escherichia* is a microorganism selected from *Escherichia coli* JSAP1b/pcDNA3 (FERM BP-6567), *Escherichia coli* JSAP1c/pcDNA3 (FERM BP-6568), *Escherichia coli* JSAP4/pcDNA3 (FERM BP-6569), *Escherichia coli* JSAP5/pGAD10 (FERM BP-6570), and *Escherichia coli* JSAP5/pcDNA3 (FERM BP-6928).

12. A method for producing the polypeptide of claim 1 or 2, comprising culturing the transformant of any one of claims 8 to 11 in a medium to produce and accumulate the polypeptide of claim 1 or 2 in the culture, and recovering the polypeptide from the culture.

13. An oligonucleotide which is selected from an oligonucleotide comprising a sequence identical to continuous 5 to 60 bases in a nucleotide sequence in any one of the DNA's of claims 3 to 5 and the DNA comprising the nucleotide sequence represented by SEQ ID NO:5, an oligonucleotide comprising a sequence complementary to the oligonucleotide, and an oligonucleotide analogue of these oligonucleotides.

14. The oligonucleotide according to claim 13, wherein the oligonucleotide analogue is selected from oligonucleotide analogues in which: a phosphodiester bond is converted into a phosphorothioate bond, a phosphodiester bond is converted into an N3'-P5' phosphoramidate bond, a ribose-phosphodiester bond is converted into a peptide-nucleic acid bond, uracil is substituted with C-5 propynyluracil, uracil is substituted with C-5 thiazoleuracil, cytosine is substituted with C-5 propynylcytosine, cytosine is substituted with phenoxazine-modified cytosine, ribose is substituted with 2'-O-propylribose, and ribose is substituted with 2'-methoxyethoxyribose.

15. A method for detecting mRNA encoding the polypeptide of claim 1 or 2, comprising using the oligonucleotide of claim 13 or 14.

16. A method for inhibiting expression of the polypeptide of claim 1 or 2, comprising using the oligonucleotide of claim 13 or 14.

17. An antibody which recognizes the polypeptide of claim 1 or 2.

18. A method for immunologically detecting the polypeptide of claim 1 or 2, comprising using the antibody of claim 17.

19. A method for immunohistologically staining of the polypeptide of claim 1 or 2, comprising using the antibody of claim 17.

20. An immunohistologically staining agent, comprising the antibody of claim 17.

21. A method of screening a compound having an inhibitory activity on binding of a polypeptide to JNK3, comprising bringing the polypeptide into contact with JNK3 and a test sample, said polypeptide comprising an amino acid sequence selected from the amino acid sequences represented by any one of SEQ ID NOS:9 to 16 or a polypeptide comprising an amino acid sequence in which at least one amino acid has been deleted, substituted or added in an amino acid sequence selected from the amino acid sequences represented by any one of SEQ ID NOS:9 to 16 and being capable of binding to JNK3.

22. A method of screening a compound having an inhibitory activity on phosphorylation of a polypeptide caused by activated JNK3, comprising bringing the polypeptide into contact with activated JNK3 and a test sample, said polypeptide comprising an amino acid sequence selected from the amino acid sequences represented by any one of SEQ ID NOS:9 to 16 or a polypeptide comprising an amino acid sequence in which at least one amino acid has been deleted, substituted or added in an amino acid sequence selected from the amino acid sequences represented by any one of SEQ ID NOS:9 to 16 and being capable of binding to JNK3.
23. A compound obtained by the method of claim 21 or 22 or a pharmacologically acceptable salt thereof.
24. A method of screening a compound capable of changing expression of a gene encoding a polypeptide, comprising bringing a cell which expresses the polypeptide into contact with a test sample, said polypeptide comprising an amino acid sequence selected from the amino acid sequences represented by any one of SEQ ID NOS:9 to 16 or a polypeptide comprising an amino acid sequence in which at least one amino acid has been deleted, substituted or added in an amino acid sequence selected from the amino acid sequences represented by SEQ ID NOS:9 to 16 and being capable of binding to JNK3.
25. The method according to claim 24, wherein the expression of a gene is detected by the method of claim 15.
26. The method according to claim 24, wherein the polypeptide is detected using the method of claim 18.
27. A compound obtained by the method of any one of claims 24 to 26 or a pharmacologically acceptable salt thereof.
28. An inhibitor of binding of a polypeptide and JNK3, wherein the polypeptide comprises an amino acid sequence selected from the amino acid sequences represented by any one of SEQ ID NOS:9 to 16 or a polypeptide comprising an amino acid sequence in which at least one amino acid has been deleted, substituted or added in an amino acid sequence selected from the amino acid sequences represented by any one of SEQ ID NOS:9 to 16 and being capable of binding to JNK3.
29. An inhibitor of phosphorylation of a polypeptide by activated JNK3, wherein the polypeptide comprises an amino acid sequence selected from the amino acid sequences represented by any one of SEQ ID NOS:9 to 16 or a polypeptide comprising an amino acid sequence in which at least one amino acid has been deleted, substituted or added in an amino acid sequence selected from the amino acid sequences represented by any one of SEQ ID NOS:9 to 16 and being capable of binding to JNK3.
30. An agent for preventing neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis and the like, amyotrophic diseases, such as amyotrophic lateral sclerosis and the like, ischemic diseases, brain damage due to stroke, schizophrenia, depression, epilepsy, or various immunological and inflammatory diseases, comprising the polypeptide of claim 1 or 2.
31. An agent for treating neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis and the like, amyotrophic diseases, such as amyotrophic lateral sclerosis and the like, ischemic diseases, brain damage due to stroke, schizophrenia, depression, epilepsy, or various immunological and inflammatory diseases, comprising the polypeptide of claim 1 or 2.
32. An agent for preventing neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis and the like, amyotrophic diseases, such as amyotrophic lateral sclerosis and the like, ischemic diseases, brain damage due to stroke, schizophrenia, depression, epilepsy, or various immunological and inflammatory diseases, comprising the oligonucleotide of claim 13 or 14.
33. An agent for treating neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis and the like, amyotrophic diseases, such as amyotrophic lateral sclerosis and the like, ischemic diseases, brain damage due to stroke, schizophrenia, depression, epilepsy, or various immunological and inflammatory diseases, comprising the oligonucleotide of claim 13 or 14.
34. An agent for preventing neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis and the like, amyotrophic diseases, such as amyotrophic lateral sclerosis and the like, ischemic diseases, brain damage due to stroke, schizophrenia, depression, epilepsy, or various immunological and inflammatory diseases, comprising the antibody of claim 17.

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35. An agent for treating neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis and the like, amyotrophic diseases, such as amyotrophic lateral sclerosis and the like, ischemic diseases, brain damage due to stroke, schizophrenia, depression, epilepsy, or various immunological and inflammatory diseases, comprising the antibody of claim 17.

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36. A promoter DNA which controls transcription of a gene encoding the polypeptide of claim 1 or 2.

37. A method of screening a compound capable of changing efficiency of transcription by the promoter DNA of 36, comprising bringing a test sample into contact with a transformant comprising a plasmid containing the promoter DNA and a reporter gene connected to the downstream of the promoter DNA; and measuring a translation product content of the reporter gene.

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38. The method according to claim 37, wherein the reporter gene is a gene selected from a chloramphenicol acetyl-transferase gene, a β -galactosidase gene, a luciferase gene, and a green fluorescent protein gene.

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39. A compound obtained by the method of claim 37 or 38 or a pharmacologically acceptable salt thereof.

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FIG. 1

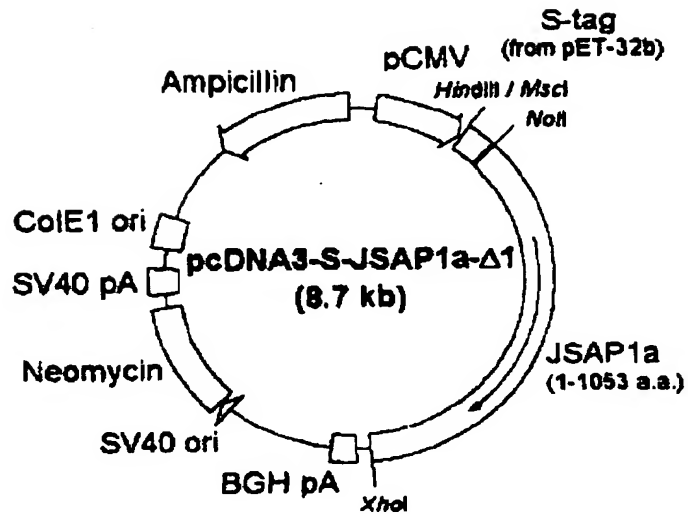


FIG. 2

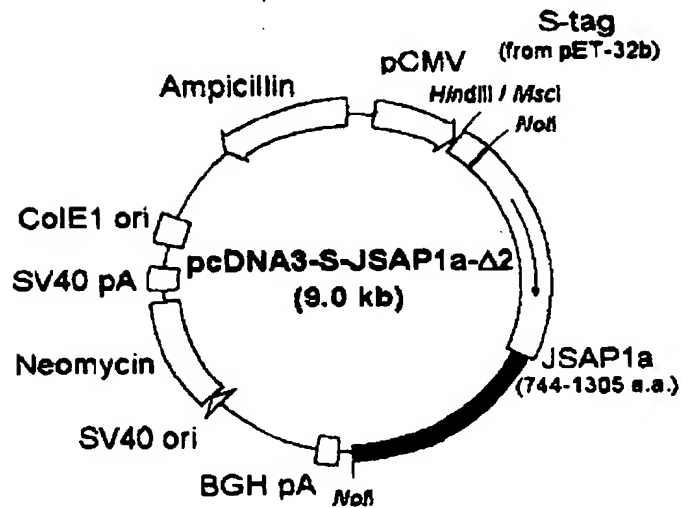


FIG. 3

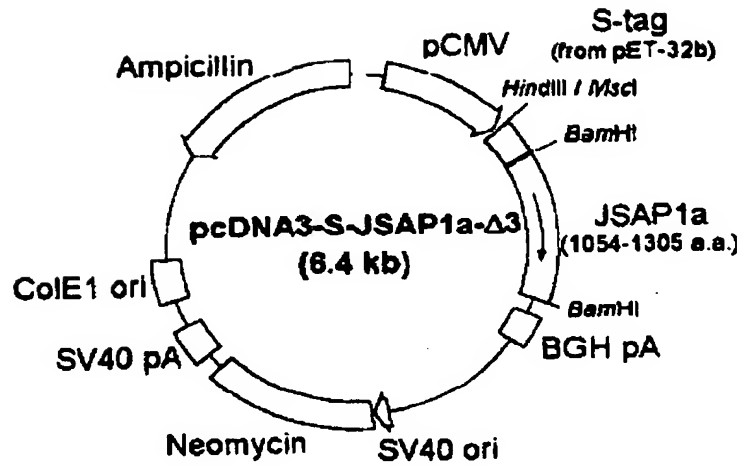


FIG. 4

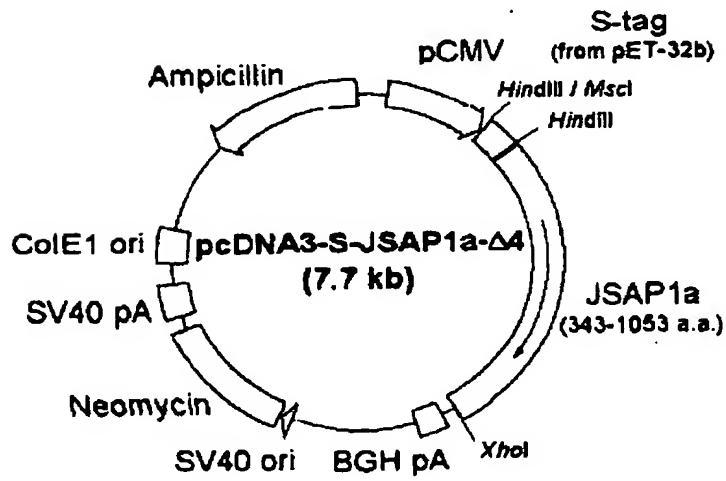


FIG. 5

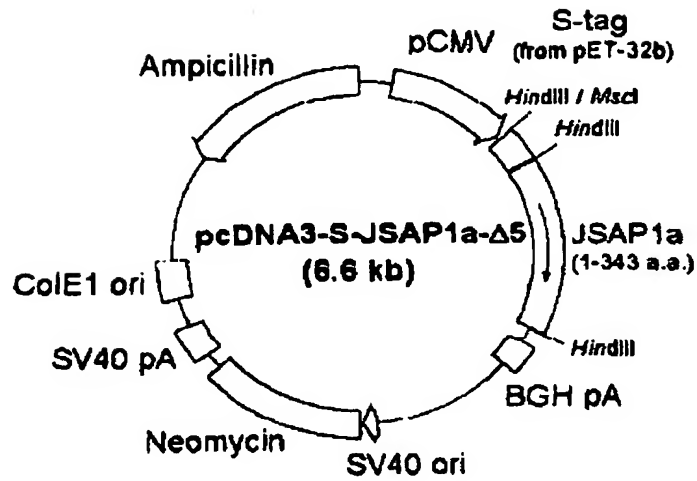


FIG. 6

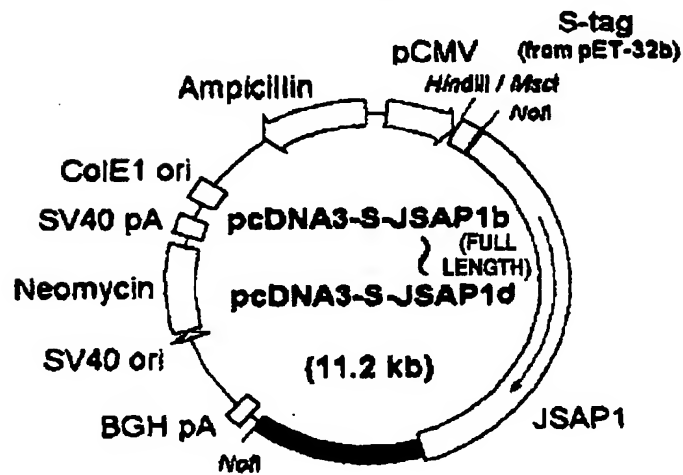


FIG. 7

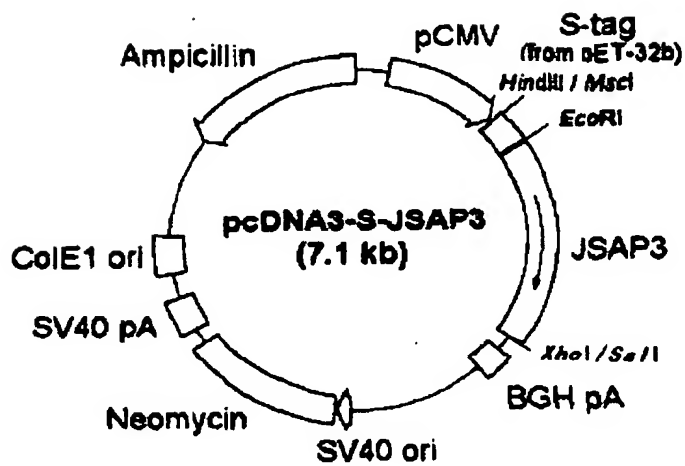


FIG. 8

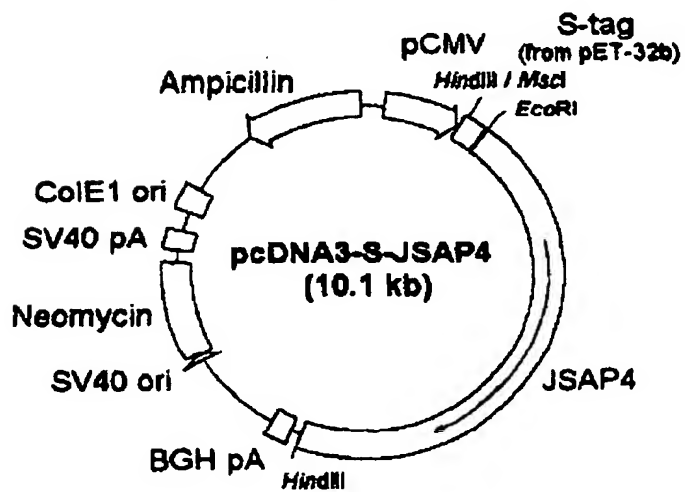


FIG. 9

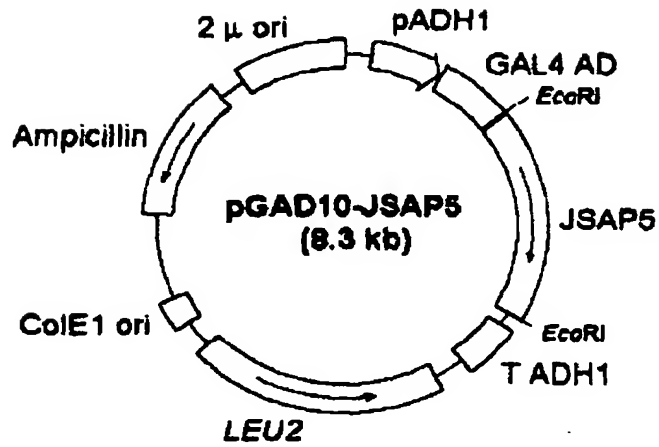


FIG. 10

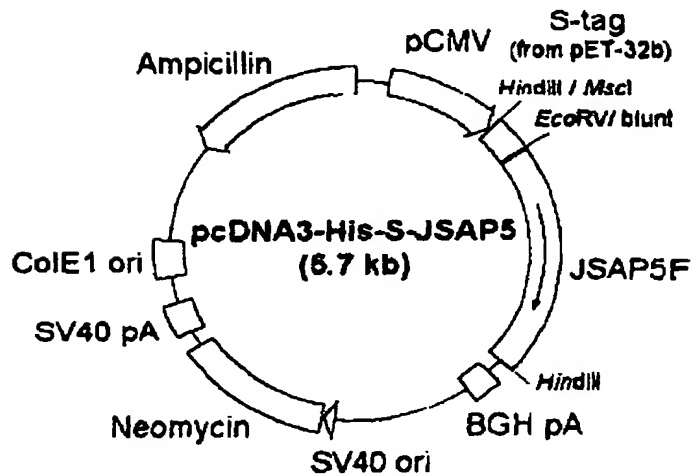


FIG. 11

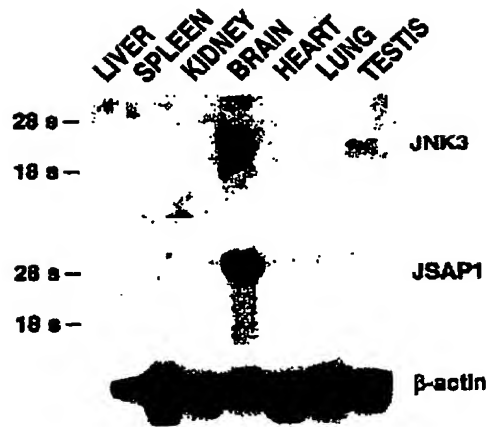


FIG. 12

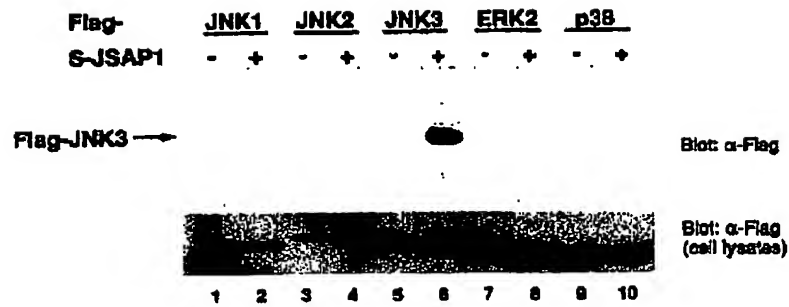


FIG. 13

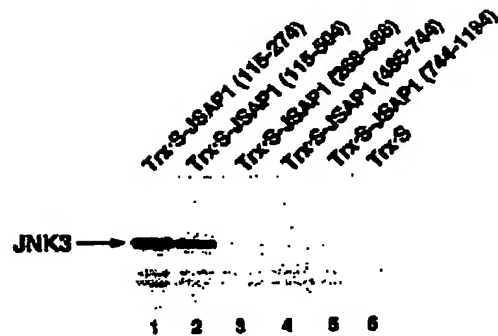


FIG. 14

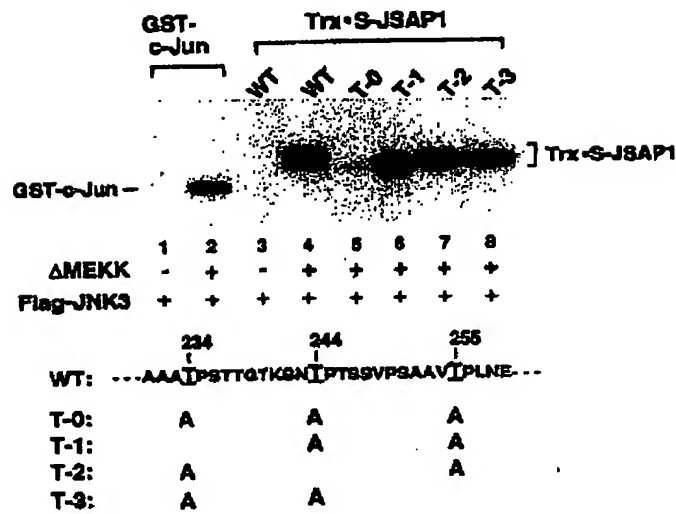


FIG. 15

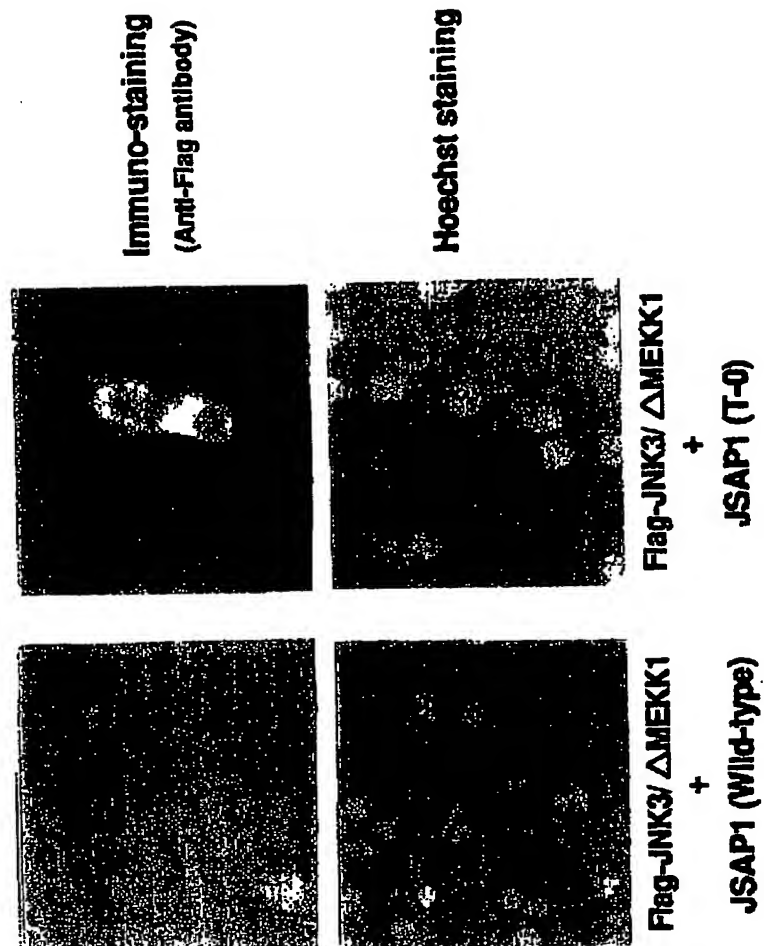


FIG. 16

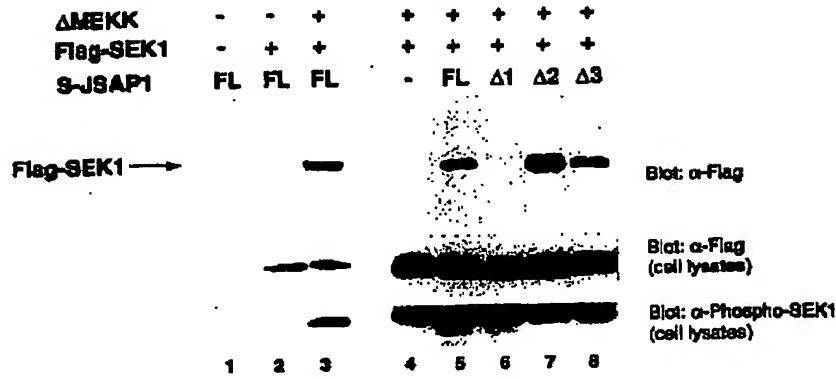


FIG. 17

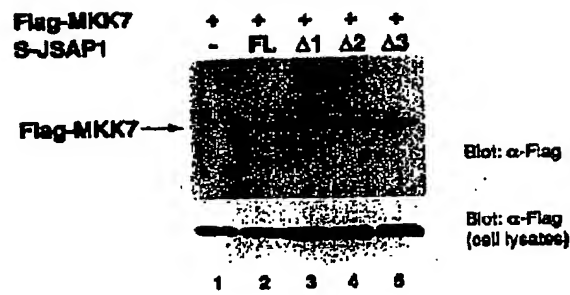


FIG. 18

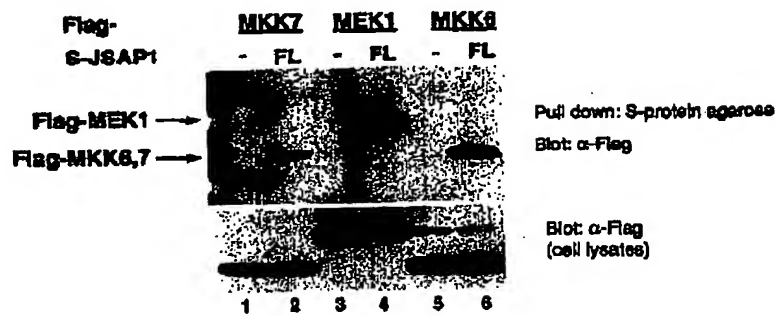


FIG. 19

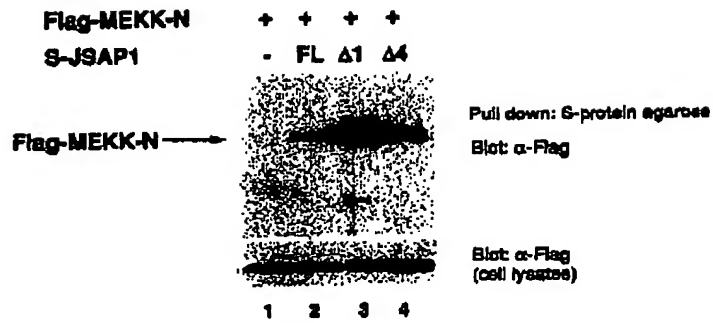


FIG. 20

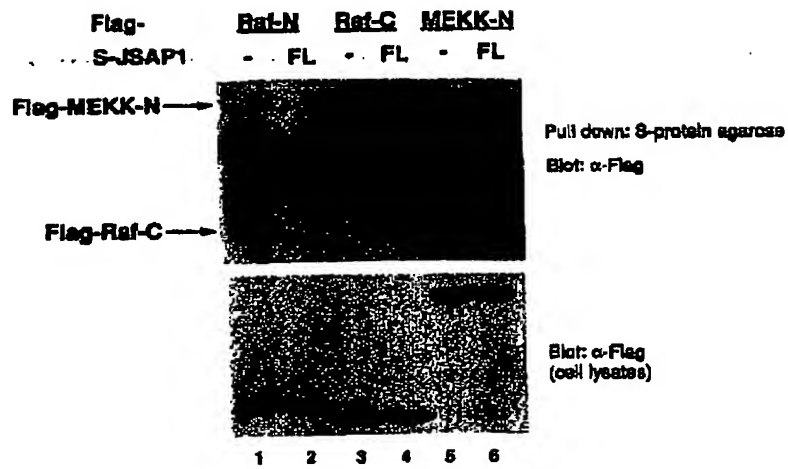


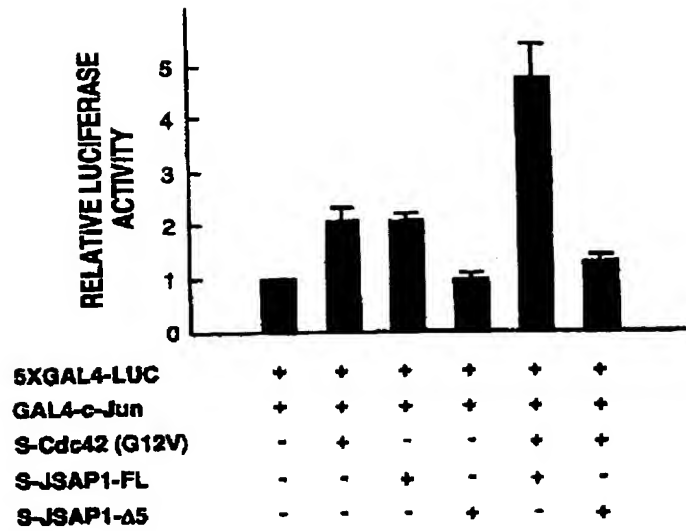
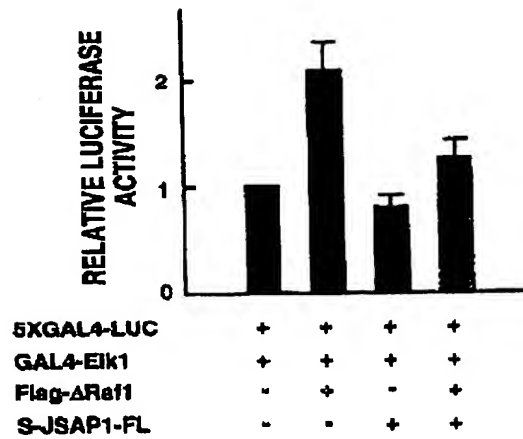
FIG. 21**FIG. 22**

FIG. 23

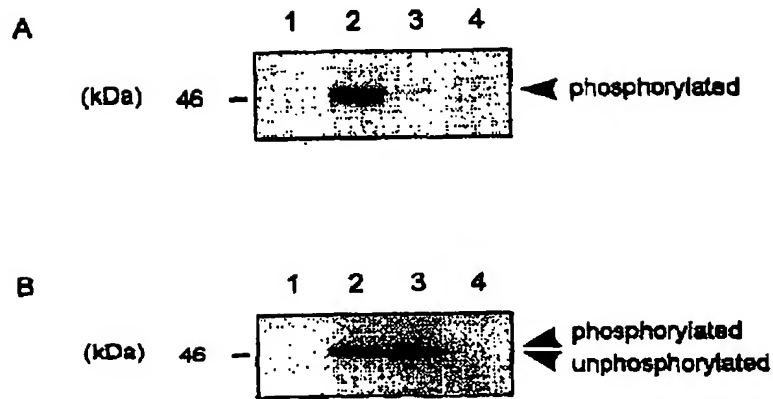


FIG. 24

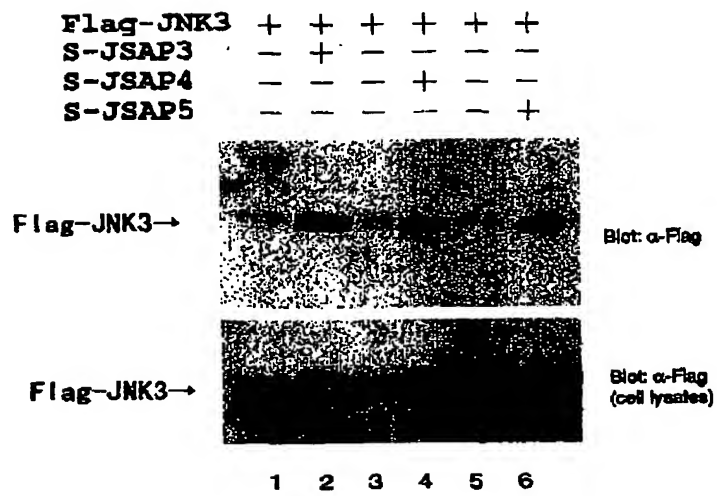




FIG. 27

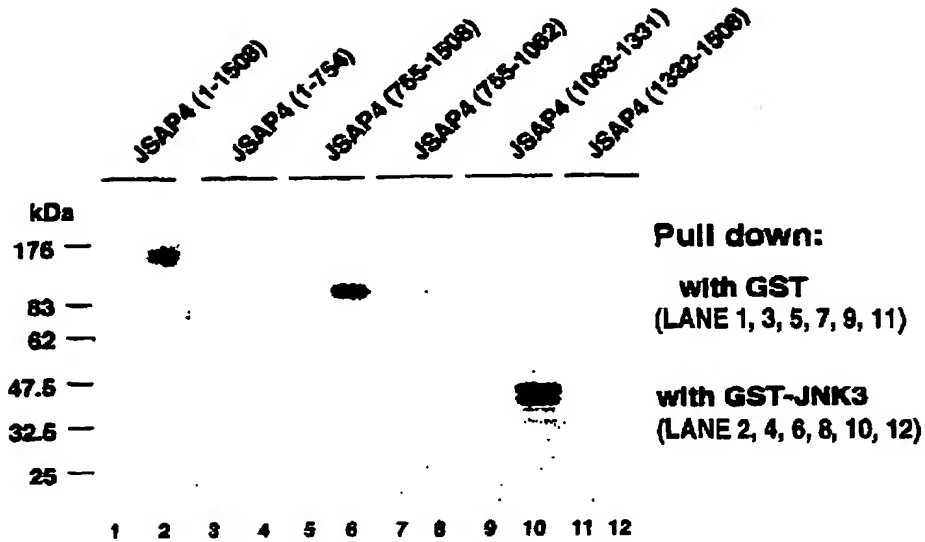


FIG. 28

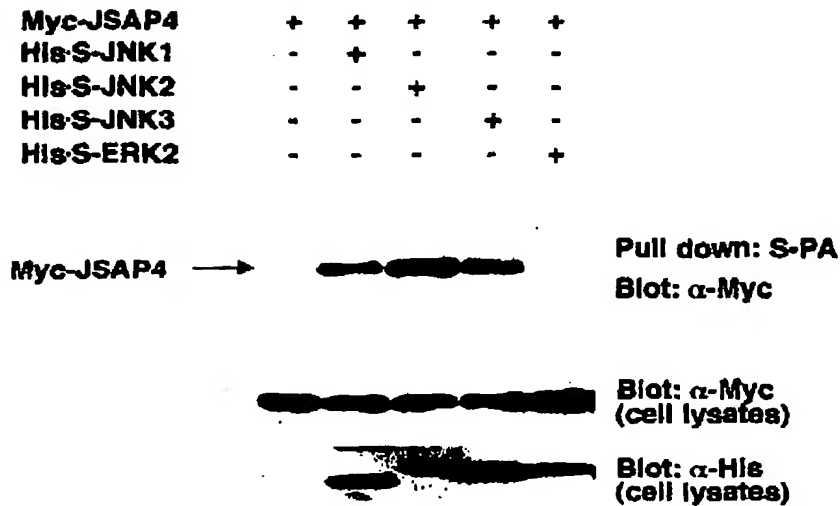


FIG. 29

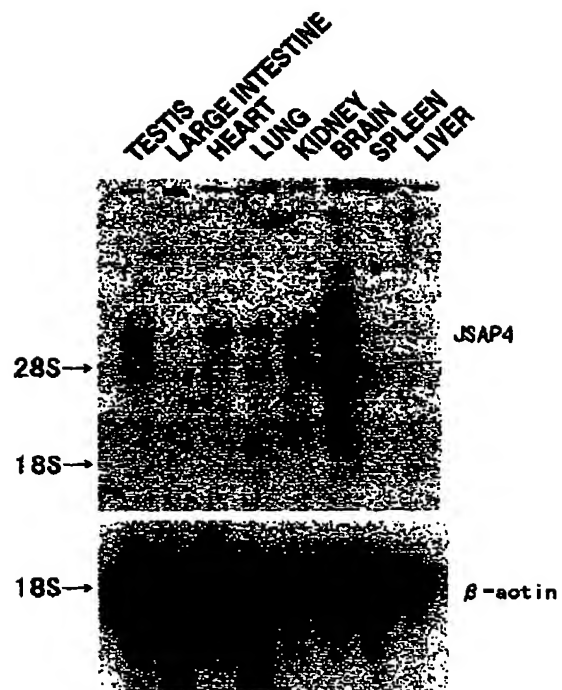


FIG. 30

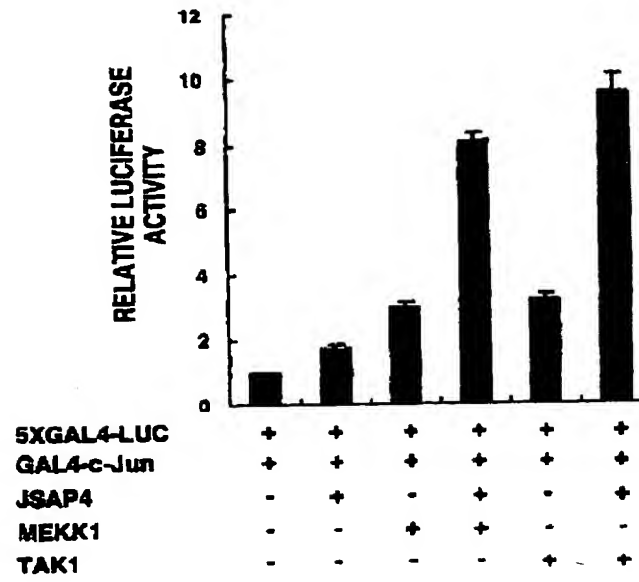
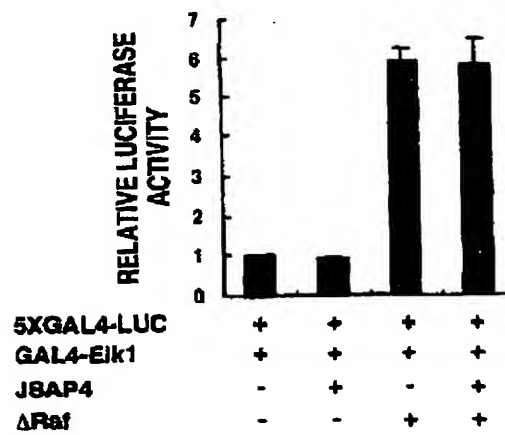


FIG. 31



INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP99/06487

A. CLASSIFICATION OF SUBJECT MATTER Int.Cl. ⁷ C07K 14/47, C12N 15/12, C12N 1/21, C12P 21/02, C12Q 1/68, C07K 16/18, C12P 21/08, G01N 33/53, A61P 25/28, A61P 25/18, A61P 25/08, A61P 37/00, C12N 15/63 // C12N 1/21, C12R 1:19), (C12P 21/02, C12R 1:19), (C12N 15/12, C12R 1:91) According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) Int.Cl. ⁷ C12N 15/00-90, C12N 1/00-5/28, C12P 21/00-08, C12Q 1/00-70, C07K 14/00-16/46, G01N 33/50-98, A61P 1/00-15/12 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) MEDLINE (STN), Genbank/EMBL/DBJ/GeneSeq, WPI (DIALOG), BIOSIS (DIALOG)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
PX PY	Ito M. et al., "JSAP1, a novel jun N-terminal protein kinase (JNK)-binding protein that functions as a Scaffold factor in the JNK signaling pathway", Mol. Cell Biol. (1999 Nov.), Vol. 19, No. 11, p. 7539-7548	1-13, 29, 30, 36 1-39
TX TY	Kelkar N. et al., "Interaction of a mitogen-activated protein kinase signaling module with the neuronal protein JIP3", Mol. Cell Biol. (2000 Feb.), Vol. 20, No. 3, p. 1030-1043	1-13, 29, 30, 36 1-39
X Y	Maria de Fatima Bonaldo et al., "Normalization and subtraction: Two approaches to facilitate gene discovery", Genome Research (1996), Vol. 6, No. 9, p. 791-806	13 14
X Y	Schaeper U. et al., "Molecular cloning and characterization of cellular phosphoprotein that interacts with a conserved C-terminal domain of adenovirus E1A involved in negative modulation of oncogenic transformation",	13 14
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Date of the actual completion of the international search 15 February, 2000 (15.02.00)		Date of mailing of the international search report 07 March, 2000 (07.03.00)
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International application No.

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Proc. Natl. Acad. Sci. USA (1995 Nov.), Vol.92, No.23 , p.10467-10471	
PX PY	Yoshioka K. et al., "A novel Jun N-terminal kinase (JNK)-binding protein that enhances the activation of JNK by MEK kinase 1 and TGF-beta-activated", FEBS Lett. (1999 Sep.) , Vol.457 , No.3 , p.385-388	1, 2, 29, 30 1-39
A	JP, 10-57074, A (Kirin Brewery Company, Limited.), 03 March, 1998 (03.03.98) (Family: none)	21-27, 37-39

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